Proliferative Vitreoretinopathy; strategies to improve anatomical and visual outcomes

PhD Thesis

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DECLARATION

I, Philip Banerjee confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated.

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ABSTRACT

Proliferative vitreoretinopathy (PVR) is the most common cause of late anatomical failure of retinal detachment surgery. Efforts to modify this vitreoretinal scarring response have so far proved clinically unsuccessful, with surgical and visual outcomes remaining poor. This work is aimed at identifying strategies to improve outcomes in eyes at high risk of PVR development following open globe trauma (OGT), and those with established PVR disease.

Two prospective clinical trials investigating the benefit of adjunctive corticosteroids in these two populations were conducted in a total of one hundred and eighty patients. Clinical and imaging data were collected over the course of approximately 3500 hospital attendances.

The Adjunct in Ocular Trauma (AOT) Trial was a two year, pilot, single-centre prospective, participant and surgeon-masked randomized-controlled-clinical trial (RCT). Forty patients requiring vitrectomy surgery following OGT were randomized to either standard (control) or study treatment (adjuncts) in a 1:1 allocation ratio. Peri-operatively, the adjunct group received intravitreal and subtenons triamcinolone acetonide, oral flurbiprofen and guttae prednisolone acetate 1%. The control group received standard care.

Primary outcome was anatomical success at 6 months and showed similar results in anatomical success with 50% (10/20) in the adjunct group, compared to 47% (9/19) in the standard group (Odds Ratio 1.11, 95% Confidence Interval 0.316-3.904). Secondary outcomes included final visual acuity, occurrence of PVR, intraocular pressure (IOP) rise, number of operations and recruitment rate. Final median visual acuity was 31 ETDRS letters in the adjunct group compared to 25 ETDRS letters in the standard group. Other secondary outcomes were similar between the two groups.

The hypothesis that an adjunctive slow-release dexamethasone implant (Ozurdex ®) could improve the outcomes of vitreoretinal surgery for established PVR was tested in the Ozurdex® in PVR Study. In this two year, single-centre prospective, participant and surgeon-masked RCT, 140 patients requiring vitrectomy surgery with silicone oil for retinal detachment with established PVR (Grade C) were randomized to either standard (control) or study treatment (adjunct) in a 1:1 allocation ratio.

Intraoperatively, the adjunct group received an injection of 0.7mg of slow-release dexamethasone (Ozurdex) at the time of (a) vitrectomy surgery and (b) at silicone oil removal. The control group received standard care.

Primary outcome measure was the proportion of patients with a stable retinal reattachment with removal of silicone oil without additional vitreoretinal surgical intervention at 6 months. Secondary outcomes included i) final visual acuity (median and ETDRS of 55 letters or better), ii) cystoid macular edema (CMO), foveal thickness and macular volume iii) development of overt PVR recurrence, iv) complete and posterior retinal reattachment, vi) tractional retinal detachment, vii) hypotony/raised IOP, viii) macula pucker/epiretinal membrane, ix) cataract, x) quality of life

All 140 patients were recruited within 25 months of study commencement; 138 patients had primary outcome data. Primary outcome assessment showed similar results in anatomical success between the two groups (49.3% vs 46.3%, adjunct vs control, (Odds Ratio 0.89, 95% Confidence interval 0.46 – 1.74, p= 0.733). Mean visual acuity at 6 months was 38.3 ETDRS letters and 40.2 letters in the adjunct and control group respectively. Secondary anatomical outcomes (complete/posterior reattachment rates and PVR recurrence) were comparable between the two groups.

Exploratory analysis suggested that the proportion of patients with cystoid macular oedema (CMO) or a foveal thickness of >300µm was lower in steroid-treated eyes compared to controls (42.7% and 47.6% vs 67.2% and 67.7%, respectively p= 0.004, p= 0.023).

Cystoid macular oedema is a secondary cause of visual loss. At 6 months following successful surgery for PVR, eyes with evidence of external limiting membrane (ELM) disruption on Spectral Domain-Optical Coherence Tomography achieve a worse visual outcome than eyes where the ELM appears preserved (p=0.006).

Provisional work using retinectomy specimens retrieved at the time of surgery in sixteen patients were studied aiming to isolate a population of Müller glia with stem cell characteristics (hMSC). This suggested that it is feasible to isolate a cell population of appropriate morphology of hMSCs, from eyes with advanced PVR. These cells survived up to ten weeks in culture but eventually terminally differentiate.

The work in this thesis has shown that corticosteroids do not modify the vitreoretinal scarring response sufficiently to improve anatomical outcomes at 6 months. Further work is required to improve the outcome in eyes with PVR. Adopting visual acuity as a primary outcome, may be a plausible design in future vitreoretinal trials.

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ABBREVIATIONS

AC	Anterior Chamber
ACIOL	Anterior Chamber Intraocular Lens
ACTH	Adrenocorticotrophin Hormone
AE	Adverse Event
AMP	Adenosine Monophosphate
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AOT	Adjuncts in Ocular Trauma
AR	Adverse Reaction
ASCOT	Adjunctive Steroid Combination in Ocular Trauma
ASR	Annual Safety Report
BCVA	Best Corrected Visual Acuity
BETT	Birmingham Eye Trauma Terminology
BLQ	Below Level of Quantification
BRB	Blood Retinal Barrier
BRVO	Branch Retinal Vein Occlusion
C_3F_8	Perfluoropropane
CA	Competent Authority
CA	Carbonic Anhydrase
CF	Counting Fingers

CFT	Central Foveal Thickness
CI	Chief Investigator
CI	Confidence Internal
CIZ	Cone Interdigitation Zone
C_{max}	Concentration (maximum)
CMO	Cystoid Macular Oedema
CMT	Central Macula Thickness
CRALBP	Cellular Retinaldehyde-Binding Protein
CREB	Cyclic AMP Response Element Binding
CRF	Case Report Form
CRF	Clinical Research Facility
CRO	Contract Research Organisation
CRVO	Central Retinal Vein Occlusion
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CTU	Clinical Trials Unit
DAPI	4’,6’-diamino-2-phenylindole
DEX	Dexamethasone
DMC	Data Monitoring Committee
DMEM	Dulbecco’s Modified Eagle’s Medium
DMO	Diabetic Macular Oedema
DNA	Deoxyribonucleic Acid

EC	European Commission
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
EL	Ellipsoid Layer
ELM	External Limiting Membrane
EMEA	European Medicines Agency
ERM	Epiretinal Membrane
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
ETDRS	Early Treatment for Diabetic Retinopathy Study
FGF	Fibroblast Growth Factor
FITC	Fluoroscein Isothiocyanate
FT	Foveal Thickness
FTMH	Full Thickness Macular Hole
GABA	Gabba-aminobutyric Acid
GAT	Goldman Applanation Tonometry
GCP	Good Clinical Practice
GFAP	Glial Fibrillary Acidic Protein
GILZ	Glucocorticoid-Induced Leucine Zipper
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Response Element

GP	General Practitioner
HM	Hand Movements/Hand Motions
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
ILM	Internal Limiting Membrane
IL-10	Interleukin- 10
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IOFB	Intraocular Foreign Body
IOP	Intraocular Pressure
IQR	Interquartile Range
IS	Inner Segment
ISF	Investigator Site File
ITT	Intention to Treat
IVTA	Intravitreal Triamcinolone
LDH	Lactate Dehydrogenase
LMWH	Low Molecular Weight Heparin
MA	Marketing Authorisation
MAPK	Mitogen-activated protein kinase phosphatase
MEH	Moorfields Eye Hospital
MHRA	Medicines and Healthcare products Regulatory Agency
MIA	Mean Intensity Adjusted

MMP	Matrix Metalloproteinases
MPP	Massive Periretinal Proliferation
MPR	Massive Preretinal Retraction
MRC	Medical Research Council
mRNA	Messenger RNA
MS	Member State
Main REC	Main Research Ethics Committee
MV	Macular Volume
MVR	Massive Vitreous Retraction
NHS R&D	National Health Service Research & Development
NP	Not Possible
NR	Not Reported
OCT	Optical Coherence Tomography
OGI	Open Globe Injury
OGT	Open Globe Trauma
ONL	Outer Nuclear Layer
OR	Odds Ratio
OS	Outer Segment
OTS	Ocular Trauma Score
PAX	Paired Box Protein
PCR	Polymerase Chain Reaction
PDGF	Platelet-Derived Growth Factor

PEDF	Pigment Epithelium- Derived Factor
PEI	Penetrating Eye Injury
PFCL	Perfluorocarbon Liquid
PI	Principal Investigator
PIL	Participant Information Leaflet
PIS	Participant Information Sheet
PK	Pyruvate Kinase
PPV	Pars Plana Vitrectomy
PVD	Posterior Vitreous Detachment
PVR	Proliferative Vitreoretinopathy
QA	Quality Assurance
QC	Quality Control
R&D	Research and Development
RAPD	Relative Afferent Pupillary Defect
RCT	Randomised Control Trial
RD	Retinal Detachment
REC	Research Ethics Committee
RMC	Research Management Committee
RNA	Ribonucleic Acid
RPE	Retinal Pigment Epithelium
RRD	Rhegmatogenous Retinal Detachment
SAR	Serious Adverse Reaction

SAE	Serious Adverse Event
SD	Standard Deviation
SD-OCT	Spectral Domain-Optical Coherence Tomography
SDV	Source Document Verification
SF	Social Functioning
SF_6	Sulphurhexafluoride
SO	Silicone Oil
SOP	Standard Operating Procedure
SLPI	Secretory Leukoprotease Inhibitor
SmPC	Summary of Product Characteristics
SPC	Summary of Product Characteristics
SRF	Subretinal Fluid
SRM	Subretinal Membrane
SSA	Site Specific Assessment
SSAR	Suspected Serious Adverse Reaction
SUN	Standardisation of Uveitis Nomenclature
SUSAR	Suspected Unexpected Serious Adverse Reaction
TA	Triamcinolone Acetonide
Tfr-rRA	Transferrin ricin-A
TGF	Tissue Growth Factor
TIA	Texture Intensity Adjusted
TIMPS	Tissue Inhibitors of Metalloproteinases

TMF	Trial Master File
TMG	Trial Management Group
TNF	Tumour Necrosis Factor
TRD	Tractional Retinal Detachment
TRITC	Tetramethylrhodamine
TSC	Trial Steering Committee
VA	Visual Acuity
VEGF	Vascular Endothelial Growth Factor
VFQ	Visual Functioning Questionnaire
VITAN	Vitreous Analysis
WMA	World Medical Authority
5-FU	5 – Fluorouracil
5-FUR	5-Fluorourudine

LIST OF PUBLICATIONS ARISING FROM WORK DERIVEDFROM THIS THESIS

Banerjee PJ, Xing W, Bunce C Woodcock M, Chandra A, Scott RAH, Charteris DG 'Triamcinolone during pars plana vitrectomy for open globe trauma; a pilot randomised controlled clinical trial' ***Br J Ophthalmol***. 2015 Nov 6 epub ahead of print

Banerjee PJ, Petrou P, Charteris DG 'Re: Afshar et al.: Dexamethasone intravitreal implant trapped at the macula in a silicone oil-filled eye (Ophthalmology 2013;120:2748-9).***Ophthalmology*** 2014 Oct;121(10):e55-7

Banerjee PJ, Petrou P, Zvobgo TM, Charteris DG. 'Spontaneous relocation of a trapped retrolenticular slow-release dexamethasone implant (Ozurdex) in a silicone oil-filled eye of a pseudophakic patient.' ***Eye (Lond).*** 2014 Aug;28(8):1036-7

Banerjee PJ, Chandra A, Sullivan A, Charteris DG "Neurotrophic corneal ulceration after retinal detachment surgery with retinectomy and endolaser; a case series" ***JAMA Ophthalmol*.** 2014 Jun 1;132(6):750-2

Banerjee PJ, Bunce C, Charteris DG 'Ozurdex ® (a slow-release dexamethasone implant) in proliferative vitreoretinopathy; study protocol for a randomised controlled trial' ***Trials*** 2013, 14:358

Banerjee, PJ, Woodcock M.G., Bunce C, Scott R, Charteris D.G. 'A pilot study of intraocular use of intensive anti-inflammatory; triamcinolone acetonide to prevent proliferative vitreoretinopathy in eyes undergoing vitreoretinal surgery for open globe trauma; the adjuncts in ocular trauma (AOT) trial: study protocol for a randomised controlled trial.' *Trials* 2013 Feb 13 14: 42.

Banerjee PJ, Wong D, Charteris DG 'Pharmacotherapy of PVR' in Vitreous: In Health and Disease; Editor. Sebag J (Springer Science) 01/2014

Banerjee PJ, Charteris DG 'Redetachment due to Proliferative Vitreoretinopathy' in Complications of Vitreoretinal Surgery Ed Lois N, Wong D, Lippincott, Williams and Wilkins (2013)

Publications submitted at time of thesis submission -

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LIST OF ORAL AND POSTER PRESENTATIONS DERIVED FROM WORK IN THIS THESIS

ORAL PRESENTATIONS

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Open Globe Injury; Pearls and Pitfalls and Clinical Trials, INVITED SPEAKER, *Eastern Deanery of Ophthalmology* U.K, Kings Lynn (June 2015)

The Adjunctive Steroid Combination in Ocular Trauma Trial; Principal Investigators Perspective *British and Eire Association of Vitreoretinal Surgeons*, Edinburgh (November 2014)

The Adjuncts in Ocular Trauma Trial; results of a randomised controlled clinical trial *EURETINA*, London (September 2014)

Ozurdex® (a slow-release dexamethasone preparation); implant behaviour in a silicone oil-filled eye, *Greek Vitreoretinal Surgeons Society*, Athens (January 2014)

'My Retina is flat but what about my cornea?' *British and Eire Association of Vitreoretinal Surgeons*, Bristol (November 2013)

<u>SUBMITTED AT TIME OF THESIS SUBMISSION</u>

Correlation between outer retinal foveal morphology and visual outcome following repair of rhegmatogenous retinal detachment with proliferative vitreoretinopathy' *Submitted to Association for Research in Vision in Ophthalmology 2016*

POSTER PRESENTATIONS

Neurotrophic corneal ulceration after retinal detachment surgery with endolaser and retinectomy; a case series. *American Society of Retinal Specialists* Toronto (August 2013)

1 Introduction

1.1 Ocular Anatomy

The eye is a highly specialised organ of photoreception. Photoreception is the process by which light energy induces changes in the retina which result in action potentials relayed via the optic nerve to the brain where the information is processed and consciously appreciated as vision. All other ocular structures are secondary to this process and either facilitate the focussing of external light onto the retina or provide nourishment for the supporting tissues in the eye. [1]

The eye is an approximate sphere with a diameter of 25mm. It can be considered as parts of two spheres; a smaller anterior part of greater curvature than the larger posterior part. The eye consists of three layers or tunics; the tough outer layer consists of the transparent cornea anteriorly and the white sclera posteriorly; the middle vascular uveal layer is composed of the iris, ciliary body and choroid; and the inner neural layer is the retina. The coats surround the transparent intraocular media (aqueous and vitreous humor) divided respectively into anterior and posterior segments by the crystalline lens.

Figure 1.1: Schematic Diagram of Eye

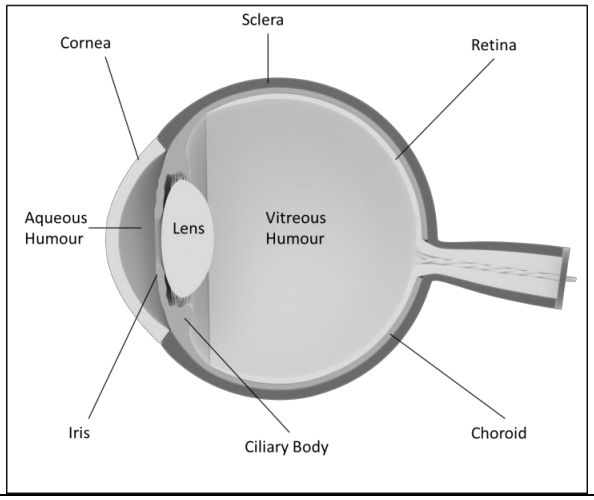


Figure 1.1 Ocular anatomy: three tunics consisting of cornea and sclera; vascular uvea (iris, ciliary body and choroid); retina (adapted from [2])

Posterior segment anatomy will be discussed in greater detail due to its relevance to this thesis.

1.1.1 Vitreous

The vitreous constitutes eighty per cent of the ocular volume and is usually about 4 mls in the average eye. It is an almost completely acellular transparent viscous fluid consisting of almost 99% water. The remainder consists of hyaluronic acid and predominantly type II collagen (although types IX and a V/XI hybrid exist in smaller quantities) and these molecular constituents are mainly concentrated in the cortical (outer) vitreous. The cortical vitreous exhibits condensations of fine collagen fibrils at its external boundaries known as the anterior and posterior hyaloid membranes.

The posterior hyaloid membrane is adherent to the internal limiting membrane (ILM) of the retina most strongly at the fovea and the optic disc. The 3-4 mm annular zone of adhesion which straddles the ora serrata is known as the vitreous base [3]. The posterior border of the vitreous base is of clinical importance and will be discussed further in this chapter (section 1.2.2).

1.1.2 Retina and Retinal Pigment Epithelium

The retina is a laminated structure consisting of the inner neurosensory retina and the outer retinal pigment epithelium. These structures are derived embryologically from two layers of ectoderm following optic cup invagination. The potential space between these layers is equivalent to the subretinal space in neurosensory retinal detachment.

1.1.2.1 *Neurosensory retina*

The neurosensory retina is a thin (150 to 400µm) transparent structure which consists mainly of neural tissue. It is comprised of photoreceptors (rods and cones), integrating cells (bipolar, horizontal, amacrine and ganglion cells) and supporting cells (Muller cells). The arrangement of these cells can be further divided into six cell layers, two layers of neuronal connections and two limiting membranes [3].

Figure 1.2: Schematic Diagram of Neurosensory Retina

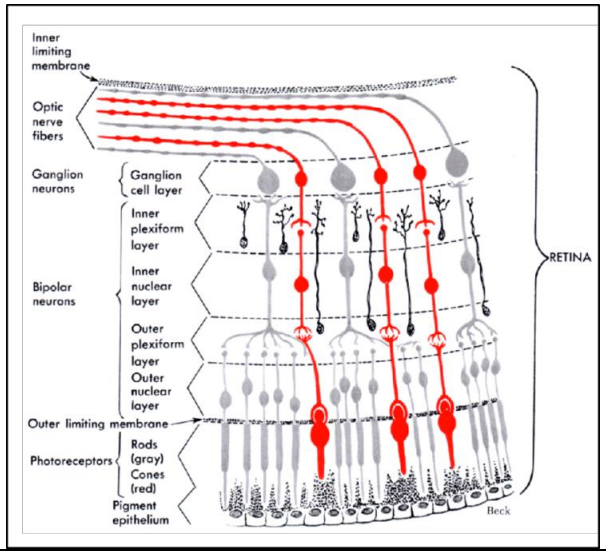


Figure 1.2. schematic representation of retinal structure demonstrating layered arrangement of neurosensory retina and attachment to retinal pigment epithelium [4]

1.1.2.2 *Retinal Pigment Epithelium*

The retinal pigment epithelium (RPE) is a continuous hexagonal monolayer of epithelial cells which extends from the margins of the optic nerve head anteriorly to the ora serrata. Here it is continuous with the pigmented epithelium of the pars plana. It has multiple properties and functions which from a crucial role in visual function. Its apices form microvilli which envelop the outer segments of the photoreceptor and adjacent RPE cells are joined by tight junctions which constitute the outer blood –retinal barrier.

1.2 Retinal Detachment

1.2.1 Categorisation

Retinal detachment (RD) is the pathological separation of the neurosensory retina from the under lying retinal pigment epithelium. It is conventionally divided into four categories depending on the primary causative pathology. The commonest is a rhegmatogenous detachment, where there is a full thickness breach in the neuroepithelium. Tractional RDs are caused by mechanical forces pulling the neurosensory retina away from the RPE. Combined tractional rhegmatogenous RDs are a combination of mechanical traction resulting in a full thickness neurosensory breach. Finally, exudative retinal detachments result from accumulation of subretinal fluid with an intact overlying neurosensory epithelium.[5]

Further expansion regarding the pathogenesis of rhegmatogenous retinal detachment will be discussed herein.

1.2.2 Pathogenesis of Rhegmatogenous Retinal Detachment

Rhegmatogenous retinal detachment occurs following a full thickness breach in the neuroepithelium allowing egress of liquid from the vitreous cavity into the subretinal space and subsequent separation of the neurosensory retina from the RPE. This breach can either be caused by dynamic vitreous traction during posterior vitreous detachment (i.e. retinal tear) or secondary to localised retinal atrophy (retinal hole). The former is more common and will be discussed in greater detail [6] .

As the eye ages the vitreous liquefies, the concentration of hyaluronic acid in the vitreous decreases[7] , thereby, allowing the collagen fibres to aggregate. [8, 9] The resultant lack of structural support causes the vitreous gel to collapse, with secondary separation of the posterior hyaloid membrane from the internal limiting membrane. This physiological 'event' occurs without adverse effect in the majority of eyes, as 63% of patients in their eighth decade have evidence of posterior vitreous detachment [10].

However, the force generated by ocular saccades is transmitted to the posterior gel and exerts dynamic traction on the retina causing it to tear in some eyes. This is usually at an area of abnormal vitreoretinal adhesion at the posterior aspect of the vitreous base. The combination of i) ongoing dynamic vitreous traction at the apex of the tear, ii) the generation of fluid currents from ocular saccadic movements and iii) gravitational force, result in recruitment of fluid from the vitreous cavity into the sub retinal space and propagation of the neurosensory detachment.

1.3 Proliferative Vitreoretinopathy (PVR)

Proliferative vitreoretinopathy (PVR) is the commonest cause of late anatomic failure in retinal detachment surgery and has a reported incidence of 5-11% of all rhegmatogenous retinal detachments [11-13]. PVR is an inflammatory process that can be considered as an exaggerated and maladapted wound healing response of the retina. It results in the formation of complex fibrocellular membranes on both surfaces of the retina, the posterior hyaloid face and in the basal vitreous. The contraction of these membranes distorts the normal retinal architecture with resultant visually detrimental sequelae. Membrane contraction can cause tractional retinal detachment, the reopening of pre-existing breaks or the formation of new ones, which may ultimately result in recurrent rhegmatogenous detachment.

Historically, based on the premise that the primary pathology was centred in the vitreous, PVR was previously referred to as massive vitreous retraction syndrome (MVR) or massive preretinal retraction syndrome (MPR). However, in order to acknowledge the role of periretinal membrane formation and pigment epithelial cell proliferation, it later became known as massive periretinal proliferation (MPP)[15].

A unifying classification system was published in 1983 by the Retina Society Terminology Committee [12] coining the phrase proliferative vitreoretinopathy (PVR), which was later updated in 1991 to the current classification system in clinical practice today [16].

1.3.1 Classification of PVR

The current classification system divides the clinical appearance into three grades of increasing severity. Grades A and B are frequently referred to as 'early' PVR. Grade A primarily consists of eyes post retinal detachment with pigment clumps in the vitreous or on the surface of the retina. Where the mobility of the vitreous and detached retina is reduced and/or the edges of the retinal break appear irregular and folded, the eye demonstrates feature of PVR Grade B.

Table 1.1: Updated Proliferative Vitreoretinopathy Grade Classification

Grade	Features
A	Vitreous haze, vitreous pigment clumps, pigment clusters on inferior retina
B	Wrinkling of inner retinal surface, retinal stiffness, vessel tortuosity, rolled and irregular edge of retinal break, decreased mobility of vitreous
CP 1-12	Posterior to equator; focal, diffuse or circumferential full-thickness folds, subretinal strands
CA 1-12	Anterior to equator; focal, diffuse or circumferential full-thickness folds, subretinal strands, anterior displacement, condensed vitreous strands

Table 1.1 is adapted from the 'Updated classification of retinal detachment with proliferative vitreoretinopathy' publication by Machemer *et al* [16]

Figure 1.3: Proliferative Vitreoretinopathy Grades A and B

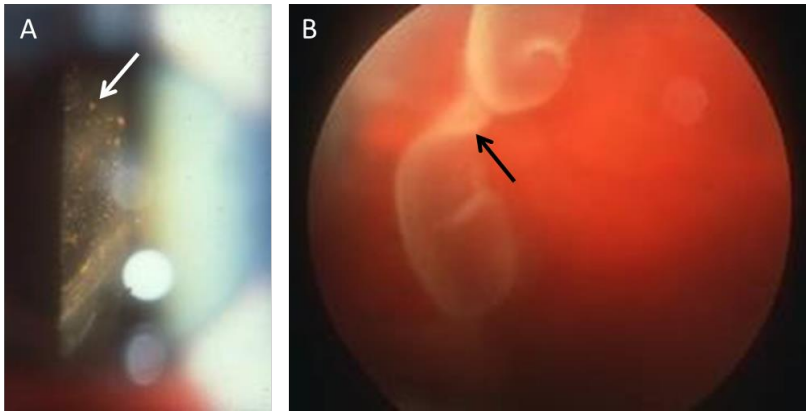


Figure 1.3 (A) Oblique slit-lamp bio-microscopic image of Grade A proliferative vitreoretinopathy (PVR). White arrow indicates clump of pigment within the vitreous cavity (B) Colour fundus photograph of retinal detachment caused by a giant retinal tear. Black arrow demonstrates rolled edge (PVR Grade B) of the retinal break

In established PVR (Grade C) formed membranes are visible on or below the retinal surface and there are diffuse or circumferential retinal folds of full-thickness. Grade C PVR is categorised into anterior and/or posterior in relation to the equator of the globe. Its extent is expressed in terms of clock hours.

Furthermore, the subdivision of Grade C PVR can be described in terms of the contraction type (Table 1.2). Focal posterior traction denotes a single starfold, which may become diffuse with confluence. Subretinal proliferations may be anterior or posterior and can appear as annular folds, linear bands and moth-eaten sheets. Circumferential contraction of membranes occurs along the posterior edge of the vitreous base thereby displacing the retina centrally and stretching the peripheral retina. Finally, there may be anterior displacement of the vitreous base with stretching or detachment of the ciliary body from anterior tractional membrane.

Table 1.2: Updated Proliferative Vitreoretinopathy Contraction Classification

Type	Location (in relation to equator)	Features
Focal	Posterior	Starfold posterior to vitreous base
Diffuse	Posterior	Confluent starfolds posterior to vitreous base; optic disc may not be visible
Subretinal	Posterior/Anterior	Proliferation under retina; annular strands near disc; linear strands; moth eaten-sheets
Circumferential	Anterior	Contraction along posterior edge of vitreous base with central displacement of retina; peripheral retina stretched; posterior retina in radial folds
Anterior	Anterior	Vitreous base pulled anteriorly by proliferative tissue; peripheral retinal trough; displacement ciliary processes may be stretched ,may be covered by membrane; iris may be retracted

Table 1.2 is adapted from the 'Updated classification of retinal detachment with proliferative vitreoretinopathy' publication by Machemer *et al [16]*

The grading of established PVR (Grade C) from fundus photographic images may be limited by the field of exposure and not accurately represent their clinical appearance. Examples of retinal detachments with their corresponding sub-classification will follow.

Figure 1.4: Proliferative Vitreoretinopathy Grade C

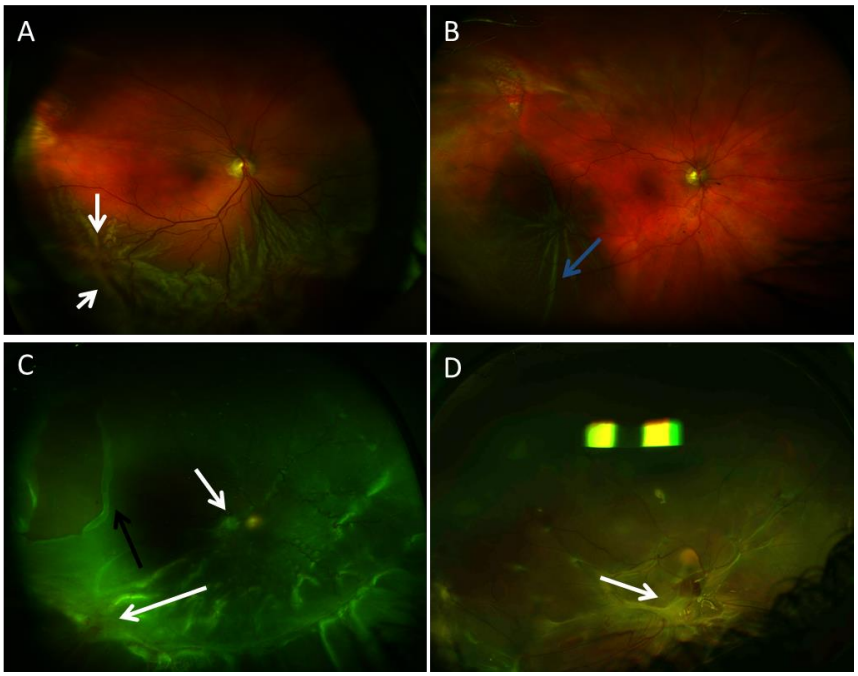


Figure 1.4 Composite figure of widefield images of retinal detachments with Grade C PVR (A) Focal posterior starfold (white arrows)of Grade CP 3 (B) Example of full thickness retinal fold (blue arrow) caused by focal starfold (C) a total retinal detachment with posterior and macular membrane and diffuse anterior membranes (white arrows), also note the rolled edge at the posterior extent of the large retinal break (black arrow), (D) extensive diffuse posterior epiretinal and subretinal membrane distorted normal retinal architecture and vasculature

Although the current classification system has served to standardize PVR terminology in clinical practice and research, it remains limited. The number, location and size of retinal breaks are not included, and many clinicians feel that grading the extent of PVR membranes in terms of clock hours limits their description to one circumferential meridian, e.g. when distinguishing linear subretinal bands from confluent sheets [17].

1.3.2 Pathophysiology of PVR

The pathophysiology of PVR is a complex sequence of events which remains incompletely understood. Rhegmatogenous retinal detachment (RRD) is considered the starting point for PVR development. Vitreoretinal scarring can be considered the result of the following components: a) blood retinal barrier (BRB) breakdown; b) cellular accumulation and proliferation; c) extracellular matrix (ECM) and collagen production with fibrin deposition; d) formed membrane contraction. Growth factors may be involved in various components of the process.

1.3.2.1 *Blood retinal barrier breakdown*

The retinal vascular endothelium and the tight junctions of the RPE comprise the blood barrier. A retinal tear results in the dispersion of retinal pigment epithelial (RPE) cells into the vitreous cavity, in addition to allowing egress of vitreous fluid into the subretinal space. The blood- retinal-barrier (BRB) breakdown which follows retinal detachment appears to have a central role in the dispersion of cells and production of growth factors which promote the further evolution of PVR [18].

1.3.2.2 *Cellular accumulation and proliferation*

Analysis of excised tissue and animal models have identified four main categories of cells in PVR membranes [19] ; i) retinal pigment epithelial cells (RPE) [20-29], ii) glial cells [20, 25-33], iii) fibroblasts [20-23, 34-38] and iv) inflammatory cells (macrophages [22, 24, 36, 37, 39, 40] and lymphocytes [41-43]).

Experimental and clinical studies have described the importance of RPE cell chemotaxis, proliferation and subsequent metaplastic differentiation. They appear to adopt a fibroblast morphology under the effect of local growth factors, although the precise trigger initiating the process in not understood.

[44] Studies have demonstrated a central role of retinal glial cell activation and extension into both epiretinal and subretinal membranes [45, 46]. The intraretinal glial response following retinal detachment without PVR has been widely described[47]. Whilst it may have a protective role in the process of neurite remodelling through growth factor production [48] , a switch in equilibrium towards glial scar formation is a key component in the pathological process of PVR development. There is growing evidence that more importance should be attributed to this intraretinal Müller cell response [49] .

Macrophages and lymphocytes may play a role in membrane formation and contraction as they migrate into the vitreous cavity and subretinal space following blood retinal barrier breakdown [48] and have been found in higher concentrations in the vitreous of eyes with PVR compared to those without. Their role is likely to involve the production of fibrogenic growth factors.

1.3.2.3 *Extracellular matrix production and fibrin deposition*

Collagen (predominantly types I and III) and the cell attachment protein, fibronectin are key components in PVR membrane formation [24, 25, 50]. Both proteins are thought to be derived from RPE and glial cells. ECM turnover and remodelling is regulated by a group of proteolytic enzymes known as matrix metalloproteinases (MMPs) and their natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs). MMPs 1,2,3 and 9 and TIMPs 1,2 and 3 have been demonstrated to be present in PVR membranes and may play an important role in membrane formation [51].

Fibrin deposition in the early phase of BRB breakdown, may add to the scaffold for complex fibrocellular membrane formation in PVR [52].

1.3.2.4 *Contraction of formed membranes*

It is the contraction of the aforementioned complex periretinal and vitreous membranes which are responsible for the clinical picture of PVR. Membrane shortening may be mediated by intrinsic fibroblastic cells, some of which have been demonstrated to contain myofilaments [21, 23, 35]. Alternative explanations suggest an RPE-collagen interaction via fibronectin bridges[53].

1.3.2.5 *Growth Factors*

Growth factors (or cytokines) may mediate the aforementioned processes of cellular activation, proliferation and contractile membrane formation. Various cytokines have been shown to be present in excised PVR epiretinal membranes and in the vitreous of eyes with PVR [54, 55]. Among them, platelet derived growth factor (PDGF), acid and basic fibroblast growth factor (FGF), transforming growth factor alpha and beta (TGF α and TGF-β), granulocyte colony stimulating factor (G-CSF) and insulin-like growth factor 1 (IGF-1) may be the most important. Some of these growth factors (i.e. PDGF, FGF and TGF) have fibrogenic activity.

1.3.3 Clinical Management of Eyes with Retinal Detachment and PVR

The principles of retinal detachment surgery with PVR share the same basic principles of managing RDs without PVR. These include the closure or sealing of retinal breaks and the complete release of pre-retinal traction. Achieving these endpoints in eyes with PVR is challenging. The addition of prevention of re-proliferation may be added as a third factor in PVR cases.

Successful repair necessitates adequate vitreous clearance with particular attention to the basal vitreous. This serves to both remove the activated cellular components as well as reduce the scaffold for subsequent contractile membrane formation. To achieve adequate anterior vitreous dissection lens removal may be required.

In established PVR (Grade C), membrane peeling is performed in order to relive traction exerted on the retinal break. This may sometimes be difficult to achieve in anterior PVR both due to access, and due to the continuity of glial cells between the retina and the fibrocellular PVR membrane [16]. With residual anterior traction, a relieving retinotomy and anterior retinectomy is necessary.

The Silicone Study Reports [56]demonstrated comparable efficacy of silicone oil and perfluoropropane gas (C_3F_8) as an intraocular tamponade in the management of established PVR. Whilst individual surgeon preference may favour one agent over the other, most cases of established PVR are managed with silicone oil [54].

1.4 Modifying the PVR Response

Although the pathophysiology of the PVR process remains incompletely understood, clinical observation and laboratory investigation have identified potential therapeutic targets for pharmacological adjuncts to act upon.

A variety of agents have been identified as potential adjuncts to modify the vitreoretinal scarring response, with corticosteroids offering the advantage of activity against multiple stages in the PVR process. Triamcinolone acetonide and dexamethasone have been the most widely studied corticosteroid agents in the management of PVR. A more detailed description of these preparations will be included in Chapters 2 and 3, respectively, where they have been adopted as the primary investigational medicinal products of two randomised controlled clinical trials.

The remainder of this section of this chapter will broadly outline the mechanisms of action of corticosteroids and both pre-clinical and clinical evidence supporting their use in eyes with PVR. Thereafter, a short summary outlining other therapeutic agents which have been investigated in the pharmacotherapy of PVR will follow.

1.4.1 Corticosteroids

Corticosteroids (or glucocorticoids) are among the most widely used therapeutic agents and their mode of administration varies depending on their target tissue. They are effective in many inflammatory and immune-mediated conditions. Developments in the understanding of the mechanisms of gene transcription and cell signalling in inflammation has helped to explain how they are able to modify the inflammatory pathway with resultant therapeutic effect. [57]

1.4.1.1 *Mode of action*

Corticosteroids (or glucocorticoids) are produced endogenously in the zona fasiculata of the adrenal cortex and effect a wide variety of metabolic, immunosuppressant, anti-proliferative and anti-inflammatory activities. An overview of these actions is not included in this thesis, although an explanation of the proposed mechanism of action of exogenous corticosteroid administration will be discussed herein.

In 1950, the Nobel Prize for Medicine and Physiology was awarded to Kendall and Reichstein, and Philip Hensch. The former partnership had successfully synthesised cortisol and then adrenocorticotrophin hormone (ACTH), and Hensch had described its dramatic efficacy in rheumatoid arthritis patients. [58] A short time later, this beneficial effect was extended to asthma sufferers initially via oral administration, and eventually in 1972 as inhaled corticosteroid. [59]

Relatively recent developments in the understanding of the mechanisms of gene transcription and cellular signalling in inflammation have served to explain how corticosteroids are able to attenuate the inflammatory response. [57] [60] The predominant effect of corticosteroid is to switch off multiple inflammatory genes that have been activated during the inflammatory process [58]. These genes encode for inflammatory cytokines, enzymes, receptors, proteins, chemokines and adhesion molecules.

The glucocorticoid receptor (GR) resides in the cytoplasm of almost every vertebrate mammalian cell. Cytoplasmic GRs are bound to molecular chaperone proteins such as heat shock protein -90 (hsp90) and FK-binding protein, thus protecting its receptor, and preventing its nuclear localisation.[61] Although only a single gene encodes the human GR, there are five different promoters, and the gene is translated into three messenger RNAs (mRNAs), each of which encodes two different proteins. There are two isoforms of the glucocorticoid receptor; GRα and GRβ. The GRα binds corticosteroids, whereas the GRβ binds to DNA and cannot be activated by corticosteroids. [58] Furthermore, GRβ has been shown to be transcriptionally inactive and is expressed at low levels compared to GRα. It has, however, been shown to block the effect of GRα and has been implicated in steroid resistance in asthma [62], although its functional relevance remains in question. Hereafter, the abbreviation GR will relate specifically to the GRα, unless otherwise stated.

Glucocorticoids are highly lipid soluble and readily cross the phospholipid cell membrane of the target cell. Once bound to GR, the activated complex effects its action via both genomic and non-genomic pathways.

1.4.1.1.1 Genomic action of Glucocorticoids

The activated GR-corticosteroid complex results in dissociation of the molecular chaperone proteins, thus unmasking the receptor nuclear localisation sites, and a resultant rapid transfer into the cell nucleus. Here it binds to DNA at specific sequences in the promoter region of corticosteroid-responsive genes known as glucocorticoid response elements (GRE) upon which, it affects gene transcription. Both positive and negative GREs exist, with activation of the former resulting in increased gene expression (*trans*-activation) and the latter decreased expression of genes (*cis*-repression) [63]. Genes which have been upregulated by activation of positive GREs encode a wide variety of proteins which exhibit anti-inflammatory effects. Such proteins include annexin 1 (lipocortin I), p11/calpactin binding protein, interleukin 10 (IL-10), secretory leukoprotease inhibitor 1 (SLPI), and Mitogen-activated protein kinase phosphatase (MAPK phosphatase). Additionally,

corticosteroids switch on the synthesis of proteins that affect inflammatory signal transduction pathways i.e. glucocorticoid-induced leucine zipper protein (GILZ), which inhibits both the pro inflammatory transcription factors NF-kB and AP-1. [64]

1.4.1.1.2 Non genomic action of glucocorticoids

The activated GR complex may indirectly inhibit inflammation through the interaction with transcription factors but without directly binding to DNA (non-genomic). NF-kB requires engagement of co activation molecules such as cyclic AMP response element binding protein (CRBP) which is dependant in the acetylation of their intrinsic histone. The nuclear GR complex may interfere with CBP histone acetylation, thereby indirectly inhibiting NF-kB expression. [58]

1.4.2 Corticosteroids in the pharmacotherapy of PVR

The following sections include excerpts from a published book chapter (Banerjee *et al,* 2014 [65])

Corticosteroids emerged as the first pharmacological agent to be employed as an adjunctive agent to target the scarring response. Their anti-inflammatory and anti-proliferative properties together with activity in blood-ocular-barrier breakdown reduction target key components of the PVR process. A variety of modes of corticosteroid administration have been investigated; systemic (oral), periocular and intraocular (by direct injection or via the infusate).

1.4.2.1 *Preclinical Evidence*

The effects of glucocorticoids on cellular proliferation both *in vitro* and *in vivo* have been widely described in non -ocular experimental cell systems [66]. Exploiting the anti-proliferative properties of corticosteroids served the basis of many experimental and early clinical cancer studies prior to the introduction of modern chemotherapeutic agents.

In the context of the vitreoretinal scarring response, an intravitreal injection of corticosteroid was first reported to significantly reduce experimental PVR in rabbits by Tano *et al* in 1980. In this animal model, tractional retinal detachment (TRD) in rabbits was significantly reduced from 57% to 24% and from 84% to 34% after a single injection of 1mg dexamethasone or triamcinolone acetonide, respectively [67, 68]. This effect was later confirmed using 2mg of intravitreal triamcinolone acetonide (IVTA) in an experimental rabbit model of PVR, where the rate of TRD was reduced from 90% to 56% [69].

Peri-ocular administration of methylprednisolone (10mg) was shown to reduce experimental complicated RD from 87% to 14%, also showing a reduction in cellular proliferation within the vitreous microenvironment [70]. More recently, this anti-proliferative effect has been confirmed by a significant reduction in human retinal pigment epithelial cell proliferation *in vitro* following a dose-dependent treatment of unpreserved triamcinolone acetonide [71].

An experimental rabbit model published in 2007 investigated the retinal toxicity of commercially available Kenalog® (triamcinolone acetonide). Albini *et* al compared dark -adapted electroretinography of 10 rabbit eyes injected with 4mg/0.1ml of IVTA with the fellow eyes serving as controls. At 2 and 12 weeks post injection, they found no discernible difference in the electroretinograms of either group. Histological analysis of

sacrificed animals showed no abnormality in the retinal architecture and the absence of intraretinal gliosis confirmed by immunohistochemistry.

The effect of the IVTA (2mg/ml) on the intraretinal glial proliferative response was investigated in an experimental retinal detachment model in rabbits. At day 3, eyes treated with intraviteal triamcinolone showed a significant reduction in the absolute number of proliferating glial cells compared to controls. Cellular proliferation was measured using the thymidine analogue 5-bromo-2'deooxyuridine (BrdU). The BrdU positive cell count was 21 (+/- 2 SEM) cells/mm in control eyes compared to 7(+/- 1.5 SEM) in IVTA –treated eyes.

Figure 1.5: Modification of Intraretinal Glial Proliferation with Triamcinolone Acetonide

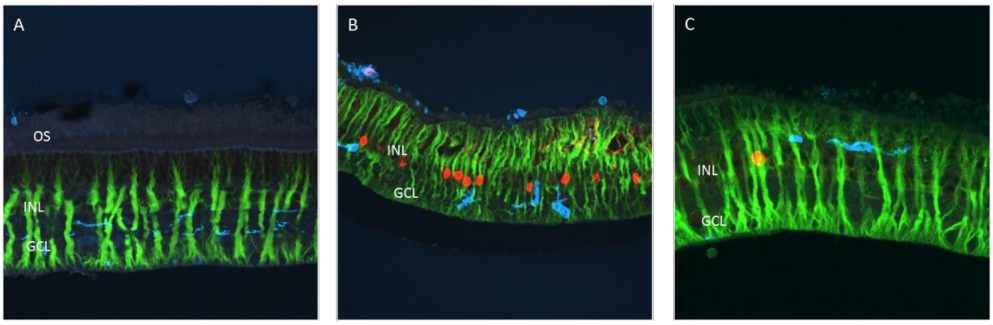


Figure 1.5 Reproduced with permission granted by D Charteris. OS, outer segments of photoreceptors, INL, inner nuclear layer, GCL, ganglion cell layer (A) normal rabbit retina showing Muller cells staining positive for anti-Vimentin (green) and microglia positive for anti-isolectin B4 (blue). (B) and (C) Day 3 post experimental retinal detachment in rabbit model. (B) proliferating Muller glial cells are demonstrated staining positive for anti-BrdU (red) in the inner nuclear layer. (C) eye treated with 2mg/0.05ml of triamcinolone on detachment induction shows reduction in absolute number of anti-BrdU +ve proliferating glial cells (mean of 21 cells/mm retina controls to 7 cells/mm retina in IVTA eyes)

An experimental study investigating the use of the slow-release dexamethasone implant, Ozurdex®, was recently described in a rabbit PVR model [72]. Interestingly, Kuo *et al* found no difference in the expression of a marker of reactive gliosis, glial fibrillary activated protein (GFAP), at day 7 or 14 between treated and untreated eyes. The clinical severity of PVR appeared comparable between the two groups. However, a significant reduction in the expression of TNFα (an inflammatory cytokine) was observed in the steroid group along with a trend towards lower expression of IL-6. Kuo *et* al acknowledged limitations of their animal PVR model in that the rapid induction of vitreoretinal fibrosis was not reflective of the indolent process clinically. Furthermore, sub-therapeutic concentrations of the corticosteroid at the investigated time-point may have limited its effectiveness.

1.4.2.2 *Clinical Evidence*

The clinical application of corticosteroids as adjuncts to vitreoretinal surgery was first reported by Koerner *et al* in 1982. They concluded that effect of oral prednisolone on postoperative retinal fibrosis did not match that of experimental intravitreal triamcinolone [73]. An infusate containing dexamethasone showed a trend towards a reduction in PVR re-proliferation and a reduction in hypotony, but did not achieve statistical significance. A reduction in blood-ocular-barrier breakdown (as measured by laser-flare photometry) was reported using subconjunctival dexamethasone (10mg) 5-6 hours prior to scleral buckle surgery [74].

Jonas *et al* opened the door to the clinical investigation of intravitreal triamcinolone (IVTA) in 2000 when they reported its potential benefit through a reduction in postoperative intraocular inflammation, without demonstrable toxicity [75] . IVTA has become the most widely investigated adjunctive corticosteroid, clinically. Its safety profile has been subsequently confirmed although its benefit as a definitive therapeutic agent has yet to be consistently proven. Reduction in blood ocular barrier breakdown [76] and a proposed benefit in established PVR have been reported [77-80] although these studies were either retrospective small or non-comparative.

A large multi-centre, prospective, quasi-randomised controlled trial investigating the use of varying doses of IVTA as an adjunctive surgical tool to aid vitreous visualization, showed a significant reduction in intraoperative complications [81] with fewer retinal breaks and intraoperative retinal detachments. However, 1 year follow up failed to show a statistical difference in visual acuity or reoperation rate [82]. The absence of any long-term positive effect may lie in its use as surgical tool rather than a therapeutic injection. It is likely that concentrations of corticosteroid remaining at the end of the procedure would have been sub-therapeutic, following its removal as part of the operative procedure.

At the time of the submission of this thesis, a single prospective randomized controlled clinical trial investigating the use of triamcinolone acetonide in eyes with established PVR (Grade C) undergoing pars plana vitrectomy with silicone oil has been reported [83]. Seventy five eyes divided into two groups in a 1:1 treatment allocation ratio were investigated. The treatment group received 4mg of intravitreal triamcinolone into the oil-filled eye at the end of the procedure, whilst controls underwent standard care. There was no statistical difference in primary anatomical success at 6 months (84% in the adjunct group vs 78% in controls). The investigated secondary outcomes (visual acuity, reoperation rate, PVR recurrence, macula pucker, IOP rise) also showed comparable results between the two groups. The authors acknowledge that a positive treatment effect may have been masked by a higher than expected primary success rate in the control group. This, together with inadequate numbers and no obvious sample size justification may have resulted in an underpowered study.

More recently, Koerner *et al* have published earlier work on the use of systemic oral prednisolone [84] and its effect on cellophane maculopathy in 220 consecutive eyes undergoing scleral buckle surgery for primary RRD. They reported significantly fewer cases of cellophane maculopathy in the steroid group 27%, 24% and 20% compared with 42%, 47% and 39% in the control group at 1, 3 and 6 months, respectively. They concluded that oral corticosteroids may have a prophylactic effect against the early

stages of PVR, but affirmed the need for larger RCTs to confirm whether the observed effect could be extended to advanced PVR.

Local corticosteroid administration is usually preferable over systemic use, as it achieves significantly higher intraocular concentrations [85] and avoids the unwanted systemic side effects.

1.4.3 Non-steroidal anti-inflammatory drugs in the pharmacotherapy of PVR

Non-steroidal agents, like corticosteroids, are of therapeutic value in vitreoretinal scarring through their anti-inflammatory properties and subsequent reduction in blood ocular barrier breakdown. They have been less widely investigated than corticosteroids due to their reduced potency and have most commonly been adopted in combination with other agents.

1.4.3.1 *Pre-clinical Evidence*

Meclofenamate and indomethacin were first shown to inhibit RPE cellular proliferation in cell culture in 1984 [86], but were not subsequently investigated as single therapeutic agents, presumably due to their inability to compete with corticosteroids as realistic treatment options. However, in combination with 5-FU in a sustained-release preparation, a significant reduction in both the presence and severity of post-traumatic experimental PVR in rabbits was reported [87].

1.4.3.2 *Clinical Evidence*

Topical indomethacin in combination with routine peroperative corticosteroids was found to significantly reduce blood-aqueous barrier breakdown in patients undergoing extracapsular cataract surgery [88] as well as decrease postoperative inflammation [89]. No clinical trials have investigated the use of non-steroidal anti-inflammatories in patients with PVR.

For completion, a brief outline of other therapeutic agents which have been investigated in the modification of PVR. They will be described in sequence followed by the preclinical and clinical evidence which supports their use.

1.4.4 Fluoropyrimidines

The fluoropyrimidines are a family of antimetabolites which modify protein synthesis and are more commonly used as a chemotherapeutic agent in solid tumours of the gastrointestinal tract. They act by (a) binding to and inhibiting the enzyme thymidylate synthetase, and (b) causing coding errors in protein translation through RNA incorporation.

1.4.4.1 *Preclinical Evidence*

5 Fluorouracil (5-FU) was first reported to reduce experimental traction retinal detachment (TRD) in non vitrectomised rabbit eyes in 1982. The TRD rate of 73.6% in control animals was reduced to 31.5% following a single intravitreal injection of 5-FU [90]. This effect was later replicated in vitrectomised eyes with repeated daily intraocular injections 0.5mg for 7 days [91]. A sustained-release preparation containing 1mg of 5-FU, reduced TRD rates from 89% in controls to 11% in an experimental animal PVR model [92]. Co-drug preparations containing 5-FU and either dexamethasone or triamcinolone have also been shown to reduce the severity and progression of experimental PVR in non-vitrectomised rabbits [93, 94].

1.4.4.2 *Clinical Evidence*

A prospective non-comparative pilot study of 22 patients with established PVR were treated intraoperatively with additional intraocular and periocular 5-FU. Sixty per cent achieved final reattachment at 6 months. The therapy was considered to be well-tolerated, non-toxic, and superior to reported standard care at the time [95]. This was subsequently confirmed in a prospective randomized controlled trial using 10mg of intravitreal 5-FU on completion of vitrectomy surgery [96]. Although a trend towards better vision in the treatment group was observed, the macula re-attachment rate was also lower compared to controls (60% vs 77%).

More recently, 5-FU has been investigated in combination with low molecular weight heparin (LMWH) in three prospective randomised controlled clinical trials [11, 97, 98] which will be outlined later in this chapter.

1.4.5 Daunorubicin

Daunorubicin, or daunomycin, is a chemotherapeutic agent of the anthracycline family previously used in combination therapy to treat haematological malignancies. It inhibits cellular proliferation by inhibiting DNA replication.

1.4.5.1 *Preclinical Evidence*

Daunomycin was shown to reduce dermal fibroblast proliferation when it was first tried intravitreally in experimental PVR in 1983 [99]. After initial concerns regarding its narrow safety margin [100], it later showed promise as a potential non-toxic and therapeutic adjunct [101-105]. In a staggered regimen with intravitreal triamcinolone, it has been shown to reduce experimental TRD in rabbits, with the staggered combination found to be superior to monotherapy. However, human multi-drug resistant cells have been found in excised premacular membranes, in eyes treated with daunomycin, thereby questioning its role as clinical adjunctive agent[106]. More recently, doxorubicin (a close relative to daunorubicin) has been shown to attenuate the intraretinal glial cell response and reduce the severity of experimental PVR [107]. It may form the basis of future studies, either as a single agent, or in combination therapy.

1.4.5.2 *Clinical Evidence*

The safety profile of intravitreal daunorubicin was first shown when administered as a 7.5µg/ml intravitreal 10 minute infusion in 15 post-traumatic eyes with PVR, prior to silicone oil injection [108]. A larger non-comparative study of 68 eyes with advanced PVR reported an eventual anatomic success rate of 73% at 18 months [109].

A multicentre, prospective, randomized, controlled clinical trial study of 286 eyes with PVR grade C2 or greater undergoing vitrectomy and silicone oil exchange randomised patients to treatment with or without a 10 minute intraoperative infusion of daunorubicin (7.5 µg/mL). Primary anatomical success, was achieved in 62.7 % of patients in the treatment group compared to 54.1% in controls. Its primary outcome marginally failed to reach significance (P = 0.07). The trial did demonstrate a statistically significant reduction in the number of required reoperations within one year (P = 0.005) [110]. Further small-scale studies have since suggested a benefit [111] but it has not gained widespread acceptance.

1.4.6 Retinoids

Retinoids are vitamin A compounds and have important roles in regulating the cell proliferation and differentiation of multiple cell types throughout the body by mediating gene transcription. They are inhibitors cellular proliferation and may modify ECM and cell-mediated contraction.

1.4.6.1 *Preclinical Evidence*

Human RPE cell proliferation was significantly reduced when grown in the presence of 1microM of retinoic acid[112]. This inhibitory effect on cell proliferation was subsequently confirmed [113], in addition to a reduction in cell-mediated contraction.

Sustained drug delivery systems containing *all-trans* retinoic acid, have been shown to reduce experimental PVR from 100% to 36% in rabbit models [114] but an associated

foreign body reaction was reported. Doses of 605 micrograms and 1070 micrograms have since been found to be therapeutic and non-toxic [115, 116]. In an experimental PVR model in rabbits using silicone oil and heavy silicone oil, *all-trans* retinoic acid significantly reduced the severity of TRDs [117]. This was later confirmed with 13-cis-retinoic acid [118]. Both isomers of retinoic acid were shown to reduce proliferation of PVR membrane-derived human RPE cells [119].

More recently, *all-trans* retinoic acid has been shown to significantly inhibit RPE cell extracellular matrix production (particularly laminin beta-1) and thereby reduce cell mediated collagen contractility [120]. It may therefore offer a unique advantage as a single therapeutic agent with activity against multiple components in the PVR process.

1.4.6.2 *Clinical Evidence*

A small retrospective study compared the outcomes of 10 patients undergoing surgery for PVR with a control group of equal number. Adjunct patients received 40mg of oral 13-cis-retinoic acid twice daily for 4 weeks postoperatively. A reduction in PVR recurrence was observed , with anatomical success in 9 out of 10 patients at 8 months compared with 4 out 10 in the control group (p=0.061) at 9 months [121].

A prospective RCT of 35 patients with PVR compared 16 patients receiving 20mg of oral 13-cis-retinoic acid twice daily postoperatively for 8 weeks 19 control patients [122]. Both anatomical and visual outcomes were superior in the treatment arm compared with the control arm, with reported final anatomical success rates of 93.8% and 63.2% (p=0.047), respectively. 56.3% of adjunct patients achieved ambulatory vision compared to 10.5% in the control arm (p=0.009). Additionally, fewer patients in the treatment group developed macula pucker (18.8%) compared with the control group (78.9%) (p=0.001). Despite this positive treatment effect, retinoic acid has not yet been universally adopted clinically which may be due to concerns regarding systemic side effects of the treatment.

1.4.7 Immunotoxins

Immunotoxins are chimeric proteins consisting of a modified antibody (or antibody fragment) attached to a biological toxin fragment with its natural binding domain removed. The cell-specific antibody binds to its target thereby allowing intracellular incorporation of the toxin and a resultant cytotoxic effect. Actively dividing RPE cells have been shown to abundantly express transferrin receptors and are thus targets for anti-proliferative therapy [123, 124].

1.4.7.1 *Preclinical Evidence*

Transferrin ricin-A (Tfr-rRA) is an immunotoxin comprised of an antibody to the RPE transferrin receptor which is linked with the A chain of ricin. Ricin is a potent toxin. It has been shown to significantly inhibit both RPE cell [125-127] and fibroblast proliferation [127, 128]. In an experimental PVR rabbit model, 10% of eyes developed TRDs when treated with an intravitreal injection of 2000ng of Tfr-rRA compared with 78% of controls [129].

VEGF receptors expressed by RPE cells have also been targeted using a combination of VEGF 165 and the diphtheria toxin (DT390-VEGF165). RPE cell survival was reduced when co cultured with this molecule in a dose-dependent response [130].

At the time of submitting this thesis there have been no clinical studies conducted to investigate the use of immunotoxins as therapies for PVR.

1.4.8 Colchicine

Colchicine is a natural product sourced from the autumn crocus plant (*Colchicum autumnale)*. It may have been employed to treat rheumatism as early as 1500 BC in Ancient Egypt. Today, it remains an alternative therapeutic agent in the treatment of gout, although its narrow therapeutic window limits its use. Colchicine prevents cell proliferation by inhibiting microtubule polymerization with a resultant inhibition of mitosis.

1.4.8.1 *Preclinical Evidence*

In 1985, colchicine was first shown to inhibit fibroblast growth in an experimental model *in vitro* [131] and later shown to be a potent inhibitor of RPE cell chemotaxis [132]. Its anti-proliferative effects were subsequently confirmed in cell culture animal models, where inhibition of RPE cell proliferation and migration, at concentrations well below levels of ocular toxicity was observed [133].

Experimental TRDs in rabbits was reduced from 74% to 29.6% at 5 weeks in animals treated with oral colchicine [134]. It has also been shown to reduce RPE-cell-mediated collagen gel contraction when human RPE cells were treated with 0.01 – 1 microM of colchicine [135]. More recently, therapies where colchicine has been combined with both methylprednisolone and sodium diclofenac [136] or 5-FU [137], have shown a significant reduction in experimental TRD rate and an inhibition of human glial cell proliferation, respectively.

1.4.8.2 *Clinical Evidence*

A small prospective controlled study in patients with PVR secondary to trauma or proliferative vascular disease compared the use of oral colchicine (1.2mg daily) with placebo (Vitamin C 250mg daily). It was concluded that the safe therapeutic dose of colchicine does not inhibit PVR [138] and it has not since been clinically investigated.

1.4.9 ECM Modifiers

Collagen (Types 1 and 3), fibronectin, and deposited fibrin form key components to the extracellular matrix found in PVR membranes. Drugs that affect their production, attachment or contraction may offer benefit in the pharmacotherapy of PVR.

1.4.9.1 *Cis-hydroxyproline*

Hydroxyproline is a major constituent of collagen stability, and its synthesis can be inhibited by a proline analogue, cis-4-hydroxyproline.

1.4.9.1.1 Preclinical Evidence

Cis-hydroxyproline was shown to inhibit bovine RPE cell proliferation, collagen synthesis, attachment, and migration *in vitro*, in a dose dependent manner [139]. More recently, two sustained-release scleral implants were investigated in an experimental PVR model. TRD rates were reduced from 89% in controls to 57% in treated animals at one month [140]. This adjunct has yet to be investigated clinically.

1.4.9.2 *Matrix metalloproteinases*

Turnover and remodelling of extracellular matrix is regulated by MMPs and their natural inhibitors, TIMPs. PVR membranes have been demonstrated to contain MMPs 1,2,3 and 9 and TIMPs 1,2 and 3 [51, 141].

1.4.9.2.1 Preclinical Evidence

Prinomastat (AG3340) is a synthetic inhibitor of MMPs that has been shown to reduce PVR in an experimental rabbit model [142] and in post-traumatic rabbit eyes [143]. It has also been shown to reduce premacular membrane formation in rat eyes [144]. It has yet to undergo clinical investigation in patients with PVR.

1.4.9.3 *Heparin/Low Molecular Weight Heparin (LMWH)*

Heparin has multiple cellular effects that can potentially inhibit PVR development. It inactivates thrombin by binding to anti-thrombin, promoting thrombin-antithrombin complex formation.

1.4.9.3.1 Preclinical and Clinical Evidence

In preclinical studies heparin has been shown to reduce fibrin formation and interfere with cell-substrate adhesion by binding fibronectin It also binds fibrogenic growth factors FGF, EGF and PDGF (refer section 1.3.2.5) and inhibits RPE cell proliferation [145].

A prospective RCT investigating the effect of heparin in the infusate on post-operative fibrin formation showed a positive effect using concentrations of 10 IU/ml, but a greater tendency to intraocular haemorrhage. Lower concentrations were ineffective at reducing fibrin formation [146]. Combined heparin and dexamethasone in the infusate suggested a trend towards a reduction in post-operative PVR in treated patients, but again higher reported rates of intraocular haemorrhage [147].

The low molecular weight fragments of heparin (LMWH) have less effect on the coagulation cascade or platelet function than heparin and thus reduce the risk of haemorrhagic complications but produce a comparable antithrombotic effect [145]. Intraocular fibrin formation was markedly reduced using an infusate containing LMWH in vitrectomy/lensectomy surgery in rabbits [148].

1.4.10 Combined 5 Fluorouracil and Low Molecular Weight Heparin

The combination of LMWH with 5-FU to modify PVR development in eyes undergoing vitrectomy surgery has been investigated in three large prospective RCTs [11, 97, 98].

All three trials adopted a common adjunctive regimen as follows; an intraoperative vitrectomy infusion solution of Hartmann's containing 5-FU at a concentration of *200ug*/ml and LMWH at a concentration of 5IU/ml for one hour. Plain Hartmann's solution was used as a placebo in control patients.

The three studies investigated eyes with (i) high risk retinal detachments undergoing vitrectomy and gas exchange [97], (ii) established PVR undergoing vitrectomy and with silicone oil [98] and (iii) unselected primary retinal detachments undergoing vitrectomy with gas [11].

1.4.10.1 *High Risk Retinal Detachments*

High risk cases were identified using a previously published regression formula based on PVR risk factors [149]. In 174 patients, PVR recurrence rates were significantly lower in the adjunct group (12.6%) compared to controls (26.4%) with fewer reoperations. Furthermore, visual outcome was better in adjunct patients who developed recurrent PVR.

1.4.10.2 *Retinal Detachments with Established PVR*

A total of 157 patients with established PVR (Grade C) undergoing vitrectomy surgery with silicone oil tamponade were randomized to receive the adjunctive combination or placebo in a 1:1 treatment allocation ratio. No benefit was found in primary anatomical success or in reported secondary outcome measures (complete or posterior retinal reattachment, visual acuity, hypotony, cataract, keratopathy).

1.4.10.3 *Unselected Primary Retinal Detachments*

641 patients with unselected primary RDs undergoing vitrectomy with gas tamponade were studied in a 1:1 treatment to control allocation ratio. Primary anatomical success rates at 6 months were 82.3% and 86.8% in the adjunct and control groups, respectively. The proportion of patients who required reoperations due to PVR was 7.0% in the treatment group, compared to 4.9% of controls. Concerns of toxicity were reported in eyes with fovea-sparing retinal detachments and treated with the adjunctive regimen.

1.5 Rationale behind need for further clinical trials of adjunctive therapy in the pharmacotherapy of PVR

It is clear from the evidence outlined throughout section 1.4 that a multitude of therapeutic agents targeting various components of the PVR process have been identified as potential adjuncts in the pharmacotherapy of PVR. Promising findings from laboratory and experimental work have yet to be consistently replicated clinically. This may in part be due to the inherent limitations of animal models of retinal detachment and PVR due to cross-species variation in retinal anatomy. The rabbit model has been most frequently utilised, but, whilst (like humans) the rabbit retina is rod-dominant, it has no intraretinal vasculature, with vessels on the surface of the posterior vitreous face serving as blood supply to the inner retina. Furthermore, it only serves as a useful short-term model of RD as the rabbit retina undergoes rapid degeneration following neurosensory detachment.

Despite improvements in instrumentation and technique over the last twenty years of clinical practice, little progress has been made in terms of both visual and surgical outcomes, with the incidence of PVR remaining relatively static [48].

There therefore remains a clear need to identify an effective adjunctive therapy through the process of clinical trial investigation.

1.6 Clinical Trials; a history of evolution and regulatory milestones

In order to determine whether a novel therapy or treatment paradigm offers additional advantages over the existing treatment, a comparison is usually necessary. When the experimentation involves human subjects, the scientist or clinician has a moral obligation to preserve the human rights and dignity of the subject. Regrettably, history has proven that this fundamental moral compass has been followed to varying degrees of consistency, sometimes with devastating consequences.

Whether deviations from this path have occurred consciously or unwittingly, guidelines and frameworks have been instituted into practice. These regulatory milestones were introduced to provide researchers guidance in prioritising the protection of subjects involved in clinical research whilst preserving the scientific integrity of the research goal.

A brief overview of the evolution of clinical trials and events which triggered the implementation of key regulatory milestones will be discussed herein.

1.6.1 Ancient Babylon to streptomycin; how we got here

It may be regarded that the first documented account of a clinical trial, albeit in rudimentary form, is in The Book of Daniel in *The Bible* [150]. King Nebuchadnezzar II following the siege of Jerusalem in 587 BC ordered the capture of a group of Hebrew boys who were to be trained for the purpose of serving in the King's court in Babylon. They were instructed to be fed a rich diet of meat and wine akin to that of the palace. Daniel, one of the captured boys, refused the food as it had not been killed according to Jewish law and convinced his captor to allow himself and three others to follow a diet of pulses and water for a ten day trial period, citing a higher nutritional value of his preferred diet.

After ten days, the four boys who had not followed the King's decree were noted that '*their countenances appeared fairer and fatter in flesh than all the children which did eat the portion of the king's meat.'* The King allegedly switched those on meat and wine to the leguminous diet having observed the superior effect of those consuming the latter.

In 1025, some 1500 years later, the Persian philosopher Ibn Sina (or Avicenna, as he has become more commonly known by the latinate form of his name), completed his encyclopaedic five books entitled the Canon of Medicine. In it, he outlined how new medicines should be effectively tested in seven principles. Three of these principles are highlighted in italics with their modern day relevance overleaf:

The time of action must be observed, so that essence and accident are not confused'

This suggests that the temporal relationship between administration and effect must be related

The effect of the drug must be seen to occur constantly or in many cases, for if this did not happen, it was an accidental effect.

This observation relates to the importance of adequate sample size and predefined outcome measures

The experimentation must be done with the human body, for testing a drug on a lion or a horse might not prove anything about its effect on man

This observation is an insightful reflection on the limitations of extrapolating the findings of preclinical studies and an affirmation of the need to perform clinical trials.

In 1537, the Renaissance military surgeon Ambroise Parè, unintentionally conducted the first clinical trial of a novel medicine. When supplies of the conventional treatment of boiling oil were limited, he chose to treat battlefield gunshot wounds with a combination of egg yolk, rose oil and turpentine. He noted the superior wound healing in those patients treated with the latter and reported his findings in '*La Méthod de traicter les playes faites par les arquebuses et aultres bastons à feu* ("The Method of Treating Wounds Made by Harquebuses and Other Guns"), in 1545.

Furthermore, Dr James Lind is widely considered to be the first physician of the modern era to have conducted a controlled clinical trial. He was appalled by the high mortality rate of sailors suffering from scurvy and whilst working on a British Naval ship, the Salisbury, he planned a comparative trial to offer a cure for the then untreatable illness. His vivid description of events covers the essential elements of a controlled trial.[151]

On the 20th of May 1747, Lind described twelve patients whom he had selected as they were all suffering from scurvy, all with similar manifestations of the disease with one common diet. He divided the twelve into six pairs, with each pair ordered to supplement their diet with a different treatment as follows *' a quart of cyder a day...twenty-five drops of elixir vitriol three times a day … two spoonfuls of vinegar three times a day … sea-water… two oranges and one lemon every day … and an electary recommended by a hospital surgeon* ' . The pair who had received the Vitamin C- rich oranges and lemons recovered within six days and were fit to return to duty. [152]

Due to costs, Lind hesitated to recommend the use of citrus fruits in the treatment of scurvy despite the overwhelming observed treatment effect. Thus, the widespread recommendation of including lemon juice as a compulsory element of the seafarer's diet was not made until 50 years later by the British Navy and was soon replaced by the more cost effective lime juice. [151] Lind's report of his comparative trial is widely considered to be the precursor for modern clinical trials, in which he also included a systematic review of scurvy to date.

1.6.1.1 *Placebo*

The next historical milestone was the introduction of placebo into the control arm in 1863 by Austin Flint [153]. Previous studies had compared active treatment with the disease in its natural progression. Flint compared 13 patients in whom he had administered a placebo to 'treat' rheumatic fever with those on active therapy. He observed comparable outcomes in 12 out of the 13 patients.

1.6.1.2 *Randomisation*

There is debate as to when the concept of randomisation was first introduced into scientific trials in order to reduce treatment allocation bias. Some propose that the study by Peirce and Jastrow in 1883 was the first example. Blindfolded subjects were asked to discriminate a 1000 g weight from a 1001g or 1002g weight. The selected weight was dictated by the random drawing of a card from a specialized deck by the investigators. However, others propose that R. A. Fisher was the first to employ randomization in a study setting with his agricultural experiment (crop variation) in 1923. [154]

Nevertheless, in 1931, J. Burns Amberson published the first use of randomization in a medical trial which had been conducted five years earlier in 24 patients with tuberculosis. Patients were allocated to either active treatment or control in a 1:1 treatment allocation ratio which was determined by a coin toss. [154]

1.6.1.3 *Streptomycin Trial*

In 1948 the landmark trial investigating the effect of streptomycin in the treatment of pulmonary tuberculosis was published by the Medical Research Council [155]. This multi-centre clinical trial is considered innovative as it provided a model of meticulous design and implementation with so many key elements influencing today's protocol design.

Aside from its systematic enrolment criteria and data collection methods which offered significant advantages over the *ad hoc* nature of other contemporary research, it was the statistical design which anchored its place in history. For the first time, the investigators were able control for bias which had previously limited the scientific integrity of clinical research thus far.

Sir Austin Bradford Hill developed a randomisation scheme which replaced the preceding alternating sequence and was able to draw up a predetermined treatment allocation list which was distributed to the multiple study sites prior to recruitment. Furthermore, he introduced the concept of treatment allocation concealment to both the participants and the investigators. (I.e. double -masking).Additionally, objective outcome measures (X-rays) were assessed by masked assessors.

The Streptomycin trial of 1948 has influenced trial design and methodology to such a degree that it continues to be justifiably referred to as ground breaking.

1.6.2 Regulatory Legislation

1.6.2.1 *Nuremberg Code*

In parallel to the emergence of novel clinical trial design and methodology which was highlighted in the Streptomycin Trial, the first set of research ethics principles were established in 1947 triggered by the abhorrent and inhumane experiments conducted on prisoners during the Second World War. The Nuremburg Code was a U.S led initiative which was created to provide an international set of principles by which the scientists and doctors of Nazi Germany could be tried against. The code constituted ten key points which included the following: the necessity of voluntary informed consent in patients with legal capacity; preservation of participant safety; that human studies should only be conducted following strong pre-clinical evidence and in the absence of the availability of alternative methods; a proportionate risk: benefit ratio must exist and participants are free to discontinue their involvement at any point. [156]

The Nuremberg Code remains the most important document in the history of medical research ethics as it has served as a framework for today's principles which preserve the dignity and rights of individuals involved in human research. [156]

1.6.2.2 *World Medical Authority*

The ethical principles which were first set out in the Nuremberg Code were further developed by the World Medical Authority (WMA) in 1964 in the Declaration of Helsinki. This has undergone a series of revisions since its inception, with the most recent (seventh) in 2013.[157] The Declaration of Helsinki remains of pivotal importance to clinical trial conduct today, as it it provides the ethical foundation for ICH E6 (ICH GCP) (refer to section 1.6.2.4), the European Clinical Trial Directive (2001/20/EC) and GCP Directive (2005/28/EC) and national clinical research legislation. These directives will be discussed further in due course.

1.6.2.3 *European Community Pharmaceutical Directive*

In the mid-1950s, there was an absence of guidelines for the development, production and marketing of medicinal products [158]. Licensing authorities were yet to exist and hence the drug Thalidomide (alpha-phthalimido-glutarimide) was introduced into the German market on 1st October 1957 as Contergan, and one year later in the U.K as Distarval. Although it was initially developed as an anticonvulsant, for which it had proven to be ineffective, it was marketed on the strength of its safety profile in overdose, in that it only caused a prolonged sedation compared to the fatal side effects of its contemporaries[159]. Cruelly, the ill-fated indication in pregnancy to treat hyperemesis gravidarum (morning sickness) led to the tragic consequences of neonatal deformities [160-162] owing to its teratogenic effects unbeknown to expectant mothers.

In response to the events which shook public health authorities and the general public, the first European Community Pharmaceutical Directive (65/65/EEC) was established in 1965. This aimed to address the system failures which had contributed to the Thalidomide disasters and ensure that a medicinal product was not marketed without prior authorisation[158].

1.6.2.4 *International Conference of Harmonisation*

There then followed an emergence of multiple guidelines and legislations both nationally and globally, in order to report and evaluate the data on safety and efficacy of new medicinal products.
However, as industry sought to develop global markets, the disparity in requirements from varying member states limited its expansion. A clear need for internationally standardised guidelines was apparent in order to avoid the duplication of time-consuming and expensive test procedures whilst protecting the safety of clinical trial participants and ultimately the public.

The International Conference of Harmonisation (ICH) was therefore established in 1996 to provide an international ethical and scientific quality standard for the design, conduct, recording and reporting of clinical trials. These guidelines were to become known as the Guidelines for Good Clinical Practice (GCP).

The ICH Guidelines for Good Clinical Practice (ICH E6) were formerly introduced in 2001 as the Clinical Trials Directive (2001/20/EC) and the GCP Directive (2005/28/EC) in 2005. These European directives were subsequently adopted and enforced into UK law in 2004 (SI 2004/1031) and 2006 (SI 2006/1928), respectively.

Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki. Furthermore it aims to ensure that clinical trial data are reliable, credible and of sound scientific value.

The ICH E6 document is divided into eight sections, the second of which outlines the core principles of GCP and is reproduced verbatim as follows [163] :

1. *Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).*
2. *Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.*
3. *The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.*
4. *The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.*
5. *Clinical trials should be scientifically sound, and described in a clear, detailed protocol.*
6. *A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favourable opinion.*
7. *The medical care given to, and medical decisions made on behalf of, subjects should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.*
8. *Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).*
9. *Freely given informed consent should be obtained from every subject prior to clinical trial participation.*
10. *All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.*
11. *The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).*
12. *Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.*
13. *Systems with procedures that assure the quality of every aspect of the trial should be implemented.*

1.6.2.5 *The Medicines and Healthcare products Regulatory Agency*

The Medicines and Healthcare products Regulatory Agency (MHRA) is a government body established in 2003, which brought together the functions of the Medicines Control Agency (MCA) and the Medical Devices Agency.

The principle remit of the MHRA is to ensure that medicines and medical devices work and are acceptably safe. They assess safety, quality and efficacy of medicines and devices both prior to granting a Market Authorisation or license for clinical use and through post marketing surveillance.

They are the Competent Authority in the U.K for the regulation of clinical trials of medicines and devices, and therefore monitor compliance with statutory obligations through formal inspections [164].

Understanding the importance of clinical trial governance and its regulatory framework has proved central to the focus of much of the work outlined in this thesis.

1.7 Thesis Aims

The aim of this work was to identify strategies to improve outcomes in the management of eyes with proliferative vitreoretinopathy (PVR). Working with the team at the NIHR Biomedical Clinical Research Facility at Moorfields Eye Hospital, I primarily aimed to investigate the benefit of using local corticosteroids in the management of eyes at high risk and those with established PVR, in the clinical trial setting.

I have also aimed to identify factors associated with limited visual recovery after successful retinal detachment repair for PVR. Using optical coherence tomography (OCT), I aimed to investigate the outer retinal structure in the reattached retina, in addition to exploring OCT-derived objective markers of intraocular inflammation in this complex condition in which the inflammatory response is a key component.

Finally, I aimed to explore the proof of principle that excised tissue in eyes with PVR harbour the capability of generating a population of Müller glia with stem cell characteristics.

2 Ocular Trauma

2.1 Ocular Trauma Epidemiology

Ocular trauma is an important cause of visual impairment and blindness worldwide and a leading cause of blindness in young adult males. [165-169] In 1977, there were almost one million people in the United States living with trauma-related visual impairment, with 40,000 to 60,000 additional new cases of trauma-related blindness each year. [170] Globally it has been estimated that 1.6 million people are blind as a result of ocular trauma with 2.3 million suffering bilateral low vision and up to 19 million with unilateral blindness or low vision. [171] Ocular trauma is the commonest cause of unilateral blindness in the world today and in developing countries the high incidence of ocular trauma has extensive socio-economic costs. [171] In the UK, it was estimated that 5000 patients per year sustain eye injuries serious enough to require hospital admission and of these 250 will be permanently blinded in the injured eye [172].

In Scotland, a one year prospective observational study using the British Ophthalmic Surveillance Unit (BOSU), the incidence of serious ocular trauma (defined as that requiring hospital admission) is estimated at 1.96 per 100,000 of the population [169] with one quarter (27.2%) suffering blinding injuries (VA < 6/60). In the Scottish working population, age-adjusted incidence ratio indicated a nine fold higher risk in males. Recent European studies report incidences of 2.4 and 3.2 per 100000 per year [173, 174] for open-globe injuries (OGI) which suggests an annual incidence for the UK of between 1500 and 2000.

2.2 Ocular Trauma Classification

2.2.1 Classification by Mechanism

In 2002, Kuhn *et al* published an internationally standardised system for classifying ocular trauma such that injuries could be unambiguously described in order to facilitate the accurate interpretation of published results [175]. Figure 2.1 summarises the Birmingham Eye Trauma Terminology (BETT) system which was included in their original manuscript and will herein be used in the classification of ocular injuries throughout the remainder of the thesis.

Figure 2.1: Birmingham Eye Trauma Terminology (BETT) System

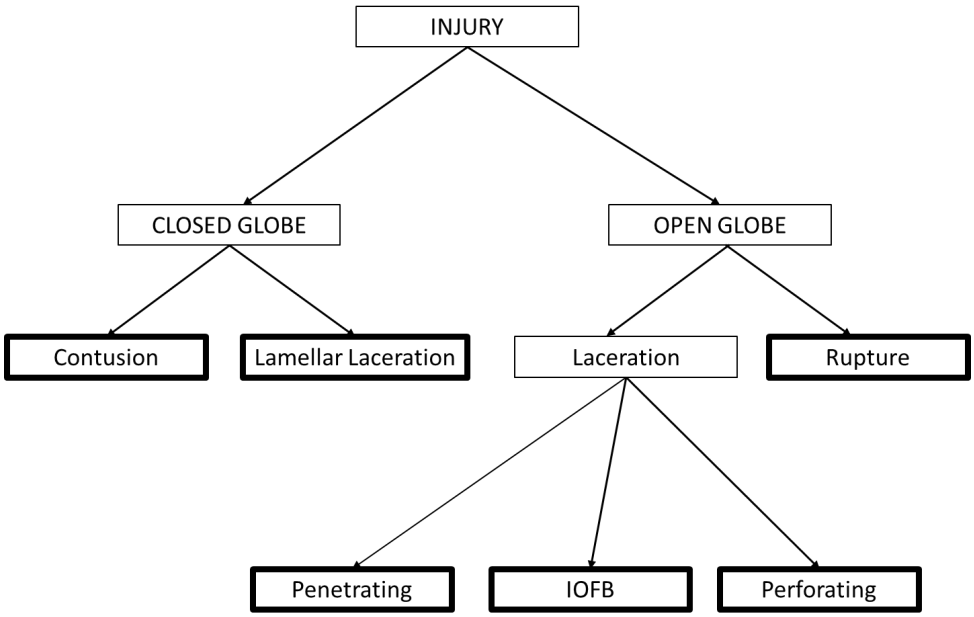


Figure 2.1 Birmingham Eye Trauma Terminology System by Kuhn *et al* [175] and shows the division of ocular injury into open globe (OGI) and closed globe injuries. Bold bordered boxes indicate preferred terminology used in clinical practice. Open globe lacerations can be further subdivided into penetrating, perforating and intraocular foreign body (IOFB) injuries.

Open globe injuries are those in which there is a full thickness injury to the eye wall and can be sub-divided into whether the injury was caused by a sharp (laceration) or blunt (rupture) appositional force. The importance of the distinction lies in the nature and amplitude of the force applied to the globe in order to generate a full thickness wound.

2.2.1.1 *Mechanisms of injury*

In general, the globe has sustained a force of significantly higher amplitude to develop a rupture compared to a laceration. It is therefore not unsurprising that the intraocular tissues are often more extensively damaged in globe ruptures when compared to an injury caused by an intraocular foreign body (IOFB).

The sequence of events culminating in globe rupture commences with globe deformation with significant antero/posterior shortening and a sudden rapid IOP rise. The globe then ruptures at the weakest site. If the eye has had previous surgery (typically with an extensive corneal wound from an extra capsular cataract extraction or penetrating keratoplasty) then this is an obvious site for the defect. If there is no history of previous surgery, the globe most commonly bursts in the weakest region i.e. circumferentially at the insertion of the recti muscles where the scleral wall is at its thinnest (Figure 2.2).

Penetrating lacerations have a single entry wound, where perforating lacerations have an additional exit wound. In practice, there may be occasions when an intraocular foreign body is caused by a high velocity blunt object e.g. a pellet or bullet and it is therefore more accurate to describe these wounds as mixed rupture with IOFB.

Figure 2.2: Mechanism of Globe Rupture

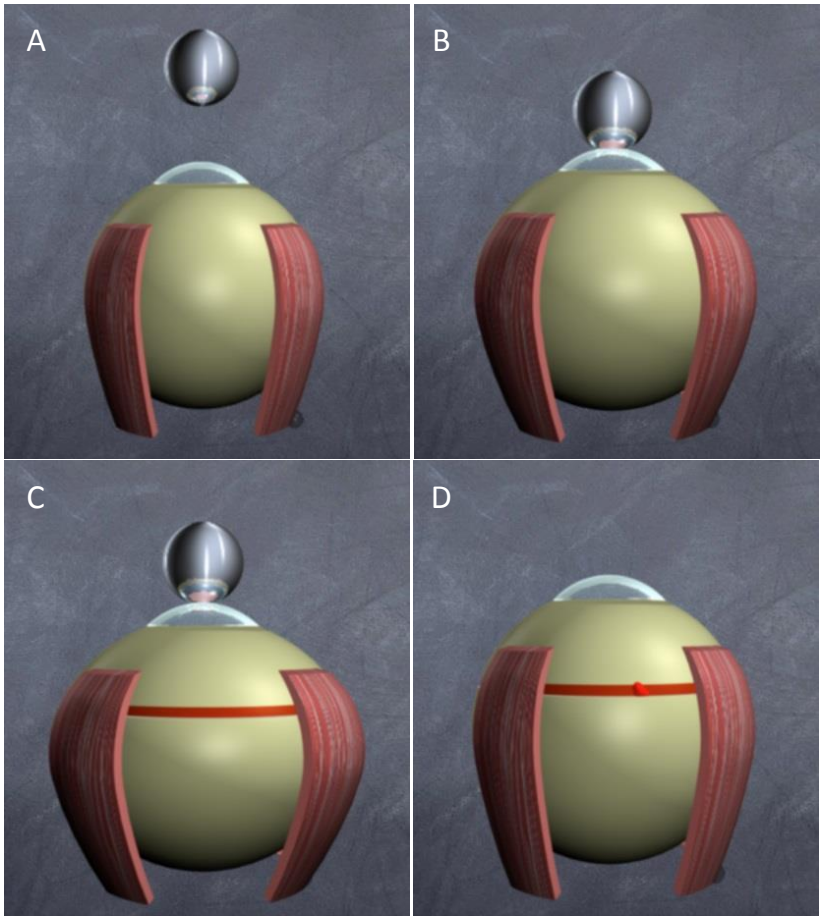


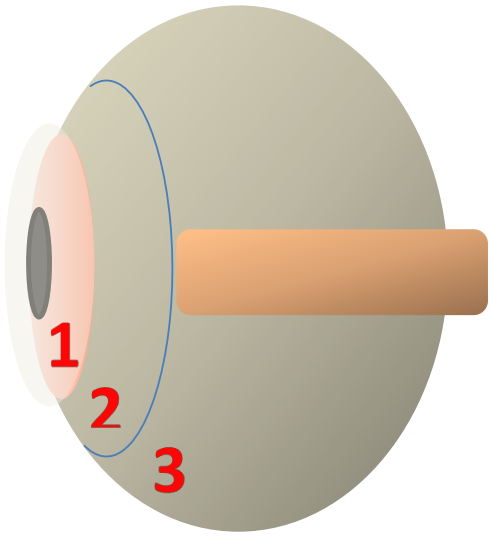
Figure 2.2 illustrates the sequence of events culminating in globe rupture (A) Blunt object towards globe of sufficient velocity to cause globe deformation and antero-posterior shortening upon impact as seen in (B). (C) sudden rapid rise in IOP sufficient to rupture globe at weakest point (red line). (D) globe rupture occurs behind insertion of recti muscles where sclera is thinnest and may result in extrusion of intraocular content (images adapted from video animation donated by Mr P Sullivan, Moorfields Eye Hospital, with permission)

2.2.2 Classification by site

In addition to classifying the injury by mechanism as mentioned above, open globe injuries can be further classified by site or extent. The eye can be divided into three zones from anterior to posterior with the boundaries dictated by ocular surface anatomical landmarks.

Figure 2.3: Schematic Eye Showing Injury Zones

Figure 2.3 demonstrates the surface anatomical landmarks to classify globe injury by site. An injury in zone 1 is entirely confined to the cornea or limbus. Zone 2 extends as far as the recti insertion. Injuries extending posteriorly to the recti insertions are classified as zone 3 injuries.



An injury that is entirely confined to the cornea or limbus is classified as a zone 1 injury. Wounds that extend up to 5mm into the anterior sclera become zone 2. In practice the recti muscle insertions are used as an estimate of 5 mm and ophthalmologists commonly use this as a surface landmark to classify such injuries as zone 2 despite the more posterior positioning of superior and medial recti. Zone 3 injuries are those which extend more than 5mm behind the limbus (i.e. behind the recti insertion).

Full thickness injuries that involve zone 3 carry a worse visual prognosis than more anterior injuries as they more commonly involve retinal tissue [176].

2.2.3 Birmingham Ocular Trauma Score

In addition to classifying the mechanism, site and extent of open globe injuries a scoring system was developed as a validated tool as an indicator of final outcome. The Birmingham Ocular Trauma Score (OTS) includes a combination of the aforementioned characteristics in order to calculate a score as follows ([177]) .

The initial presenting visual acuity is used as a starting raw point or score out of 100 as indicated in the table below.

Table 2.1: Raw Point Designation for Ocular Trauma Score

Visual Acuity	Raw Point
≥ 6/12	100
6/60 – 6/15	90
1/60 – 5/60	80
CF/HM/PL	70
NPL/Enucleation/Evisceration	60

Adapted from [177]. CF = counting fingers, HM = hand movements, PL = perception of light, NPL = no perception of light

Points are subsequently deducted from the raw point score depending on the presence of particular clinical features at presentation. Those associated with a worse visual prognosis are assigned a higher degree of importance (Table 2.2).

Table 2.2: Deduction from Raw Point to calculate Ocular Trauma Score

Clinical Feature	Reduction of Raw Point
Rupture	- 23
Endophthalmitis	- 17
Perforating Injury	- 14
Retinal Detachment	- 11
Relative Afferent Pupillary Defect (RAPD)	- 10

Adapted from [177].

Therefore an open globe injury that presents with a visual acuity of 5/60 and is classified as a rupture with a retinal detachment and relative afferent pupillary defect would be assigned an Ocular Trauma Score (OTS) as follows:

80 (raw point) – 23 – 11 – 10 = 36

Multiple studies have validated this system and found it a useful tool as a prognostic indicator of visual outcome in open globe injuries.

2.3 Posterior Segment Sequelae of Open Globe Injury

2.3.1 Retinal Detachment after Open Globe Injury

Eyes sustaining open globe injuries are at risk of severe visual loss through a variety of mechanisms. Stryjewski *et al [176]* recently published the largest retrospective review characterising the development of retinal detachment after open globe injuries. Data were available on 892 of 1036 OGIs over a twelve year period between 1999 and 2011 at a single tertiary referral unit. They reported a crude overall incidence of RD in 29% of eyes (95 % confidence interval 26-32). Unsurprisingly, the incidence was significantly higher in Zone 3 injuries (60%, 95 % CI 53-67%) compared to Zone 1 injuries (9%, 95% CI 6-12%).

Additional factors associated with retinal detachment determined on multiple variable regression analysis were the presence of vitreous haemorrhage (odds ratio [OR], 7.29; P<0.001) and poorer logarithm of the minimum angle of resolution (logMAR) visual acuity at the time of presentation after OGI (OR, 2.41 per integer increase in logMAR visual acuity; OR, 1.00-81.30; P<0.001). Interestingly, Kaplan-Meier analysis estimated that 27% (69/255) of eyes detached within 24 hours of primary open globe repair, 47% (119/255) detached within 1 week, and 72% (183/255) detached within 1 month.

The authors developed a scoring tool (RD-OGI score) to predict the likelihood of RD development following an open globe injury. This is based on the three aforementioned clinical factors at the time of primary repair, namely presence or absence of vitreous haemorrhage, highest injury zone and logMAR VA. As this has only recently been published, it has yet to be validated as a useful tool in clinical practice.

2.3.2 Proliferative Vitreoretinopathy after Open Globe Injury

Aside from the high incidence of retinal detachment after open globe trauma, eyes with a full thickness injury show an increased propensity to the development of proliferative vitreoretinopathy. It is unsurprising that the incidence of PVR varies depending on the mechanism of injury and therefore the degree of blood ocular barrier breakdown and release of secondary inflammatory cytokines.

The largest report to date was published in 1997 by Cardillo *et al* [178] . Of 1654 eyes suffering severe ocular trauma over a three year period, 347 were classified as open globe injuries. The highest incidence of PVR was observed in perforating ocular trauma, where 43% (n=13/30, $p<0.001$) of eyes developed proliferative vitreoretinopathy. 21% (n=30/145) of eyes with globe ruptures, 15% (n=15/98) of penetrating injuries and 11% (6/54) eyes with an intraocular foreign body developed PVR in their series. Other studies report a wide variety of PVR rates in open globe trauma. In a small non comparative study [179] where early (within 24 hours of injury) vitrectomy with silicone oil was performed, only 2 out of 13 cases developed proliferative vitreoretinopathy.

2.4 The Adjuncts in Ocular Trauma (AOT) Trial

2.4.1 Rationale

Section 2.3 summarises why eyes sustaining open globe injuries are a group at high risk of severe visual impairment. Retinal detachment is common and multiple surgical interventions are often necessary [176, 180]. PVR is the commonest cause of recurrent retinal detachment and visual loss in eyes with open globe trauma. It is estimated to occur in 10-45% of all OGT. [172, 178, 179, 181-186]

Although vitreoretinal surgical techniques have improved, outcomes remain unsatisfactory and that development of proliferative vitreoretinopathy (PVR) is the leading cause of this. [178, 179, 181, 182]

Section 1.4.2 has outlined the experience of corticosteroid therapy in eyes with PVR. In particular, experimental work has suggested that triamcinolone acetonide can reduce the severity of PVR if administered intravitreally [68, 187] and via periocular administration [70]. Laboratory work has also confirmed no demonstrable retinal toxicity of triamcinolone [188].

A pilot study by Wickham *et al* has shown that triamcinolone is well tolerated in PVR cases undergoing vitrectomy and silicone oil exchange and that a combination of adjuncts targeting the inflammatory component of the PVR process may be a potential treatment to prevent PVR. [189]

The Adjuncts in Ocular Trauma trial was thus developed to investigate the feasibility of conducting a prospective randomized controlled clinical trial in this demographic testing the aforementioned agents.

2.4.2 Investigational Medicinal Products

2.4.2.1 *Triamcinolone Acetonide*

Triamcinolone acetonide is a hydrophobic long-acting corticosteroid preparation which has been used off- label in clinical ophthalmic practice for many years. Ophthalmologists have experience of its periocular administration for over 50 years, with administration via the intraocular route adopted for over 30 years. It has been used to treat a variety of posterior segment ocular inflammatory pathology [190-193].

Its use as an intraocular surgical adjunctive tool for visualisation of the posterior hyaloid during pars plana vitrectomy has been well established [194]. Additionally, intraocular triamcinolone has been found to reduce postoperative inflammation following vitrectomy surgery [76]. It has been investigated specifically to determine its effect on vitreoretinal scarring (PVR) with varying success [77, 78, 83].

It has an extremely well documented safety profile with the commonest significant side effect recorded as elevated intraocular pressure (IOP) [195]. It is particularly useful in patients who have isolated ocular disease especially unilateral, providing an anti-inflammatory and anti-proliferative efficacy equal to or greater than that achieved with systemic administration while avoiding the unwanted systemic side effects of steroid use. Its effects may last up to 3 months [196] which covers the key active developmental stage of PVR, which occurs over about 6 to 8 weeks following ocular injury.

Various preparations of triamcinolone are available in the United Kingdom. Kenalog® (Squibb and sons) is the most widely used but continues to be used outside the terms of its licensed indication. Unpreserved preparations which are licensed for intraocular therapeutic use include Trivaris™ (Allergan, Incorporated) and Triescence® (Alcon, Incorporated) are now available in the U.K. but were not available at the time of study set up. Kenalog® was used as the primary IMP is this clinical trial.

2.4.2.2 *Flurbiprofen*

Flurbiprofen is an oral non-steroidal anti-inflammatory drug most commonly used to treat musculoskeletal disorders including rheumatoid disease, osteoarthritis and bursitis, but in ophthalmic practice is commonly used to treat scleritis. The rationale for its use in this study stems from evidence suggesting that non-steroidal anti-inflammatory medications given peri-operatively may limit the degree of blood-retinal barrier breakdown and have been shown to inhibit cellular proliferation in vitro. [86, 88, 89]. Furthermore, combining corticosteroids and non-steroidal anti-inflammatories has been shown to have a synergistic effect in reducing blood-ocular barrier breakdown. [89]

2.4.2.3 *Prednisolone Acetate 1%*

Prednisolone acetate 1% is an eye drop suspension used commonly in the treatment of steroid-responsive inflammatory conditions of the eye. It remains the most potent topical steroid treatment licensed for use in the U.K.

2.4.3 Purpose of the AOT trial

The primary purpose of this study was to determine the feasibility of conducting a prospective randomised controlled clinical trial using the aforementioned IMPs in this disease population. We aimed to acquire data in order to power a definitive study. Additionally, we aimed to investigate the treatment effect and toxicity of intensive anti-inflammatory agents.

2.4.3.1 *Primary Objective*

To determine whether adjunctive use of anti-inflammatory agents in eyes requiring vitrectomy following open globe trauma has an effect on anatomic reapposition of the remaining retina to the retinal pigment epithelium in the absence of an internal tamponade agent at 6 months post-surgery. This study aimed to provide sufficient data to power a definitive future study.

2.4.3.2 *Secondary Objectives:*

To determine whether adjunctive use of anti-inflammatory agents in eyes requiring vitrectomy following open globe trauma has an effect on:

i. Visual acuity at 6 months (as measured on an ETDRS Chart)
ii. Number of procedures required to achieve retinal reattachment
iii. Presence and grade of postoperative proliferative vitreoretinopathy according to the Retina Society classification on proliferative vitreoretinopathy (1991)
iv. Persistent submacular fluid found by Optical Coherence Tomography in the presence of retinal reattachment
v. Further secondary objectives include providing information regarding: rate of recruitment, case mix of ocular trauma and patient loss to follow-up rates

2.4.4 Methods/design

This was a phase 2, single-masked, single-centre, pilot, randomised, controlled clinical trial involving 40 patients. Participants were randomised into either the adjunct group (standard care with additional per-operative anti-inflammatory adjuncts) or control group (standard care with no additional treatment). Both groups received the standard surgical treatment appropriate for their eye condition and routine preoperative and postoperative treatment and care, differing only in the addition of supplementary anti-inflammatory agents in the treatment group. The study protocol has been published in Trials, an open access journal [197].

2.4.4.1 *Study Setup*

2.4.4.1.1 Funding

An unrestricted grant was obtained from the Ministry of Defence via Qinetic (Ref No. RAO 24/5/59) by the chief investigator , Professor David Charteris (DGC) and Mr Malcolm Woodcock, a co-investigator . The funding agency had no role in the management of the trial or results dissemination, but requested the provision of quarterly study reports per annum. Upon submission of the final study report, the final payment of the grant was made.

2.4.4.1.2 Regulatory Authority Approvals

Prior to participant recruitment: Moorfields Research Management Committee (RMC) approval was obtained, a favourable opinion from the Research and Ethics Committee for Wales was received (07/MRE09/60) and the study was granted a clinical trials authorisation by the MHRA. The trial was registered on the European Clinical Trials Database (EudraCT No: 2007-005138-35). The study was conducted in accordance with the International Conference on Harmonisation for Good Clinical Practice, as set out in

the European Union Clinical Trials Directive (2001) and associated UK Regulations (2004). The study complied at all times with the Declaration of Helsinki (2000). Moorfields Eye Hospital was the study sponsor and took overall responsibility of trial management. The regulatory authority approvals were obtained by DGC.

2.4.4.1.3 Case Report Form Design

Paper case report forms were designed by DGC but modified by me to ensure the efficient capture of the required dataset (Appendix 1). The study database was designed by the research department applications managers at Moorfields Eye Hospital, Richard Seeberan and Shaun Kirupairatnam. Prior to green light approval, dummy data were entered into the study database in order to test its design.

2.4.4.2 *Eligibility of Participants*

Inclusion Criteria

- All patients with an open globe injury requiring vitrectomy, either following open globe injury (OGI) or as the primary procedure itself

(OGI has previously been defined in section 2.2.1). In the event of bilateral injury, the worst injured eye (at the investigators discretion) was to be included in the study.

Exclusion Criteria

- Age < 18 or > 80 years of age
- Pre-existing Glaucoma
- Previous vitrectomy surgery to the affected eye
- Pregnant or breastfeeding females
- Previous known adverse reaction to any of the IMPs
- Inability to attend regular follow up or give informed consent

2.4.4.3 *Study Interventions*

2.4.4.3.1 Adjunct Group

Pre-operative Treatment

- **Guttae Prednisolone forte** 2 hourly for up to one week replaced any topical ant-inflammatory agents which the patient was already using, and all other ocular treatment was continued

Per-operative Treatment

- **4mg/0.1ml of triamcinolone acetonide** was injected into the vitreous cavity following closure of the scleral ports at the end of procedure
- **40mg/1ml of triamcinolone acetonide** was given as a posterior subtenons injection prior to suturing the conjunctiva
- Standard subconjunctival antibiotic injection at the surgeons' discretion

Post-operative Treatment

- **Guttae prednisolone forte** hourly for 1 week followed by a tapering regimen for 3-26 weeks thereafter at the treating clinician's discretion
- **50mg flurbiprofen** orally twice daily for 1 week
- Routine topical antibiotics and mydriatics (Guttae chloramphenicol 0.5% and Guttae cyclopentolate 1% -frequency and duration at surgeons' discretion)

2.4.4.3.2 CONTROL GROUP

Pre-operative

- No additional treatment is given. Patients were instructed to continue with their current treatment, which may have already included topical anti-inflammatory agents such as guttae dexamethasone, and topical antibiotics and mydriatics

Peri-operative

- Standard sub-conjunctival medications to include 4mg of betamethasone and 125mg cefuroxime (at operating surgeon's discretion)

Post-operative

- Routine topical antibiotics (Guttae chloramphenicol 0.5%) topical steroids and topical mydriatic (Guttae dexamethasone 0.1% and Guttae cyclopentolate 1% - frequency and duration at operating surgeon's discretion)

2.4.4.4 *Schedule of assessments*

2.4.4.4.1 Informed Consent

Informed consent was taken by me or the chief investigator prior to any study-specific interventions were performed (Appendix 3). Adequate time was provided prior to signing of the consent form which in the majority of circumstances was greater than 24 hours after issuing the participant information leaflet (PIL). Where emergency surgery was required necessitating the enrolment within this time, we ensured that patients had been provided adequate time to reach their decision. This was documented in the source documents (hospital clinical notes).

2.4.4.4.2 Screening

Screening assessments were carried out by me in order to confirm eligibility.

2.4.4.4.3 Baseline Assessment

Baseline assessments were performed by me within 2 weeks prior to the scheduled operation date. Data including: patient demographic; site, nature and extent of ocular injury; and a full slit lamp ophthalmic examination was included. Where posterior chamber assessment was directly possible, retinal attachment status, presence and Grade of PVR and spectral domain OCT of the macular was performed where possible. Where media opacity precluded a direct fundal assessment, B scan ultrasonography was used to document retinal attachment status, and parameters regarding grade of PVR and foveal thickness and volume were deemed unrecordable.

2.4.4.4.4 Follow-up assessment schedule

Post-operative study visits did not differ from the routine schedule for vitreoretinal procedures at the study site i.e. day 1, 10 days, 4-6 weeks, 3 months and 6 months. The time window allowed around these scheduled visits were as follows: Day 10 (+/- 3 days), Week 4-6 (+/- 7 days) Months 3 and 6 (+/- 14 days). I performed >95% of clinical assessments. At each scheduled post-operative study visit, a full ophthalmic assessment was completed to include slit lamp biomicroscopy (+/- indirect binocular ophthalmoscopy when required) and parameters including ETDRS visual acuity, applanation tonometry, anterior segment assessment and retinal attachment status recorded. Spectralis domain optical coherence tomography was used to document the presence of subretinal fluid within the macular field.

In patients in whom silicone oil was used as a tamponade agent, its routine removal was not considered a re-operation and routine subsequent follow up was followed until the patient returned to the study visit schedule. Other vitreoretinal surgical interventions (excluding posterior epiretinal membrane peel) over the trial period

were considered reoperations and recorded as such. Postoperative visits related to reoperations, or any other attendances outside the study visit schedule, were recorded as 'unscheduled visits'. CRFs identical in composition to the study scheduled visit CRF were completed and included in the data analysis on completion of the study.

Following the final study visit at 6 months, participants were discharged back to the care of their General Practitioner. Participants requiring ongoing ophthalmic care continued to be followed up under their admitting consultant.

2.4.4.5 *Randomisation*

Upon enrolment into the trial, patients were randomised to either the adjunct group or control group from a non-stratified pre-randomised list of 40 study IDs held by the trials pharmacist. Participants were allocated to the lowest unused study ID. Out of hours (i.e. weekends and bank holidays), the next study ID in sequence was kept in a sealed envelope in a secure location on site when access to the trials pharmacist was limited.

2.4.4.6 *Masking*

This was a single masked study to the participants. Although the pre-operative and post-operative regimens differed, participants were not informed to which group they had been allocated. It was not possible to mask the investigator, as the primary IMP, intravitreal triamcinolone, can sometimes be visible on posterior chamber assessment. The operating surgeon was masked to the randomization of the participant, until the end of the procedure, to avoid any bias regarding surgical management.

2.4.4.7 *Outcome Measures*

2.4.4.7.1 Primary Outcome Measure

The primary outcome measure was anatomic reapposition of the remaining retina to the retinal pigment epithelium in the absence of an internal tamponade agent at 6 months post primary vitrectomy surgery

2.4.4.7.2 Secondary Outcome Measures

Analysis of the following secondary outcomes at 6 months post primary vitrectomy surgery were performed:

i. Best Corrected Visual acuity using (ETDRS Chart)
ii. Number of procedures required to achieve retinal reattachment
iii. Presence and grade of postoperative PVR [16]
iv. Proportion of patients with persistent submacular fluid found by Optical Coherence Tomography in the presence of retinal reattachment.
v. recruitment rate
vi. retention rate
vii. case mix of ocular trauma

2.4.4.8 *Adverse Events and Safety Reporting*

Safety reporting adhered to the sponsor's standard operating procedures and means were in place to monitor, record and report adverse events in line with the MHRA guidelines. An external Data Monitoring Committee was established and agreed to adhere to a trial-specific charter. They were scheduled to meet six to twelve monthly or on *ad hoc* basis with a remit of monitoring the safety of the trial participants.

Adverse events (AE) were recorded and classified in terms of severity, causality and seriousness. Expected adverse event included: cataract, raised intraocular pressure, hypotony, sterile hypopyon, retinal detachment, uveitis and further surgery.

Unexpected adverse events included endophthalmitis and systemic illness.

2.4.4.9 *Trial Size and duration*

A power calculation to determine sample size was not performed as it was not required to meet the study objectives. A total of 40 patients was deemed a feasible number over the study period and expected to provide sufficient data to power a definitive study.

An internal audit of the incidence of open globe trauma at the study site provided data estimating an expected recruitment rate of 2.2 cases per month. This projected that the required recruitment target would be achieved within 18 months and completion of the trial within 24 months.

2.4.4.10 *Statistical Analysis*

The statistical analysis plan was written in advance of the data analysis in conjunction with the trial statistician and was approved by the Trial Steering Committee and the Data Monitoring Committee. Data analysis adhered to the CONSORT guidelines for randomized controlled trials. The time taken to recruit patients is reported together with the number of patients who failed to provide outcome data. Baseline characteristics of the two groups were compared to assess the adequacy of randomization.

2.4.4.10.1 Primary Endpoint Analysis

An odds ratio of patients in whom anatomical retinal attachment remained at 6 months post primary vitrectomy between the 2 treatment groups was reported with 95% confidence intervals. Since power is low, it must be acknowledged that this treatment effect may be imprecise.

2.4.4.10.2 Secondary Endpoint analysis

Secondary endpoint analyses comprised summary statistics for secondary outcomes by treatment group.

2.4.5 Results

2.4.5.1 *Recruitment*

The trial opened for recruitment on 12th September 2011 and the first patient was recruited on 22nd September 2011. The final patient was recruited on 3rd June 2013. During the first six months, the actual recruitment rate was well ahead of the projected target, but was followed by a marked plateau for a near equivalent period. The study completed recruitment approximately 10 weeks behind schedule. There did not appear to be a seasonal variation in available cases, and a retrospective review revealed one missed eligible case within the first six months and four missed eligible cases in the penultimate month.

Figure 2.4: Recruitment Line Graph

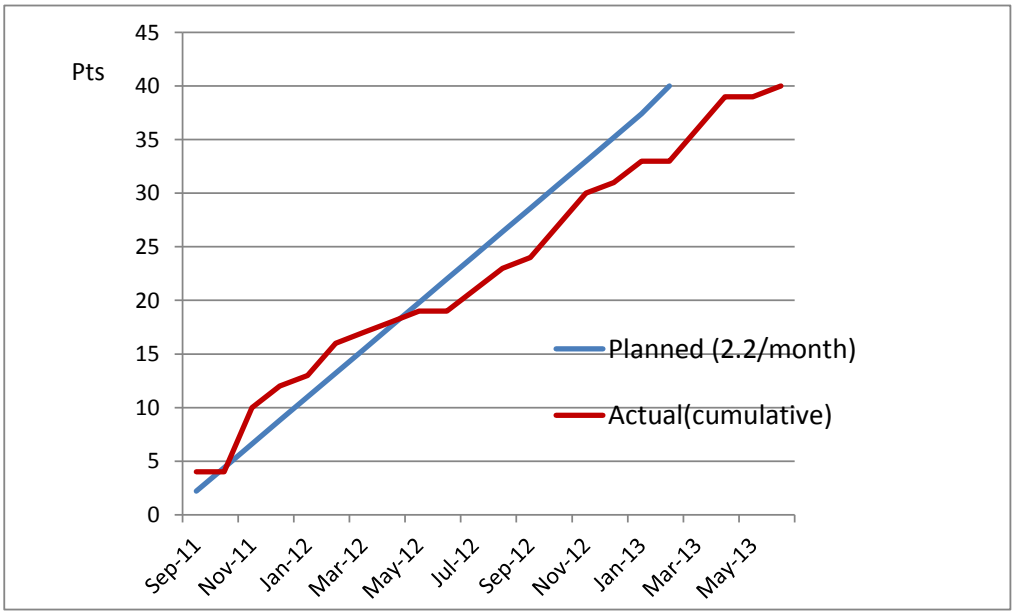


Figure 2.4 demonstrates that, initially, recruitment rates were well ahead if target. However, owing to a four month plateau occurring in the mid study period, recruitment was completed 10 weeks behind the projected completion. Missed eligible cases in the final two months contributed to the delay in recruitment.

2.4.5.2 *Study Consort Flow Diagram*

Figure 2.5 displays the consort flow diagram. Forty five patients suffering open globe injuries were identified as proceeding to pars plana vitrectomy surgery and screened for eligibility. One patient elected not to proceed with secondary vitrectomy surgery and four patients were ineligible due to pre-existing glaucoma (n=2) and aged over eighty years old (n=2). The remaining forty eligible patients elected to participate in the trial and were recruited within 21 months of the study commencing. There was a high enrolment rate (100%) once screened as eligible. This compares favourably to previously published studies where the median reported rate of enrolment was 90% [198].

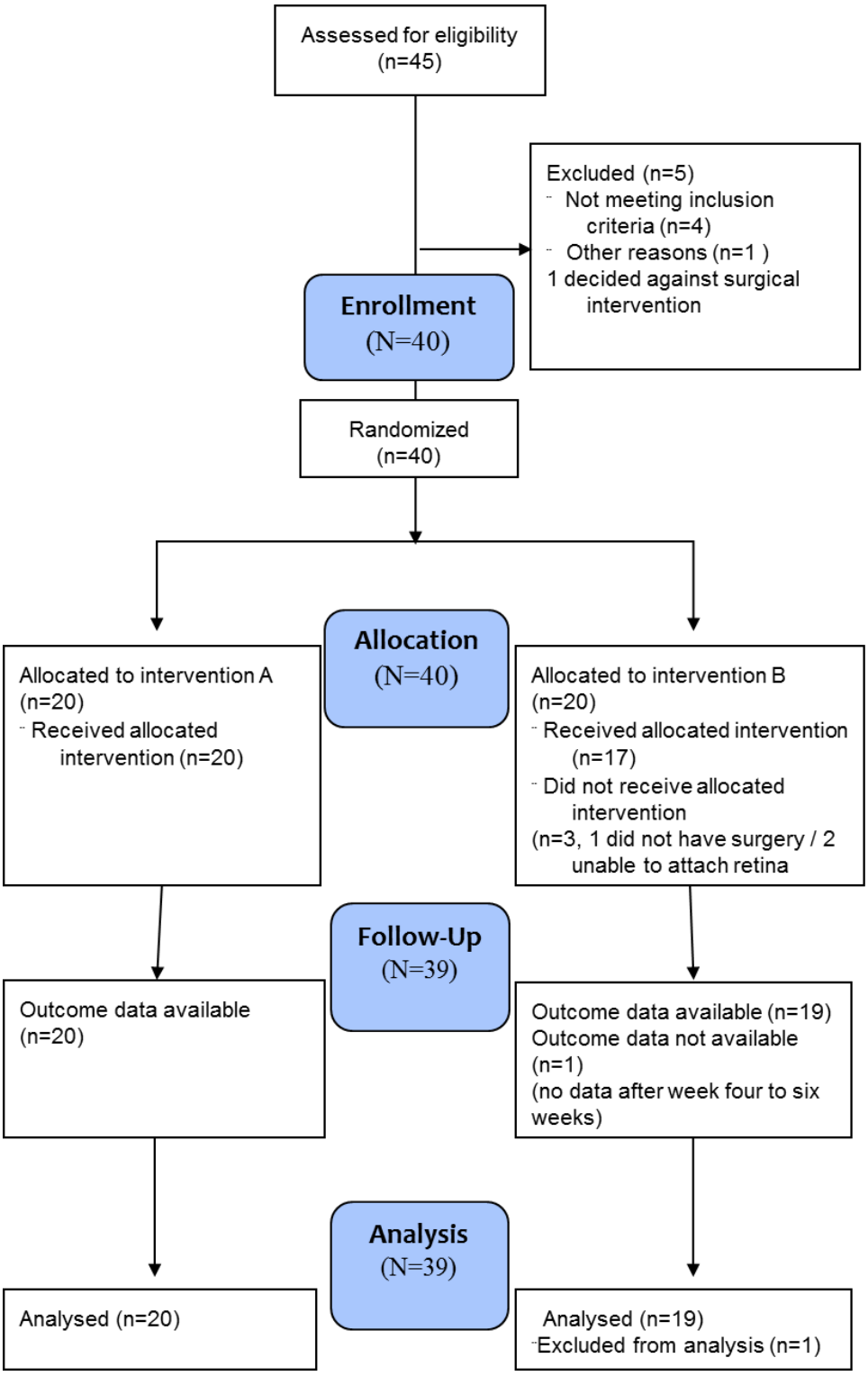
All patients in the adjunct group received their allocated intraocular, periocular and topical treatment as outlined in the treatment protocol. Oral flurbiprofen was omitted from the postoperative regimen in three out of the twenty patients in the adjunct group (15%) owing to medical contraindications to its use. This was in accordance with the study protocol.

2.4.5.3 *Participant Retention*

Thirty eight of the forty participants were retained in the study until the primary and secondary outcome assessment time-point at six months. One patient in the control group failed to attend for follow up visits after the 4-6 week assessment and was therefore excluded from the final analysis. One patient in the adjunct group attended for an additional visit at 4 months and was then subsequently lost to follow up. Their clinical condition was deemed stable and likely representative of the findings at 6 months, and therefore, data collected at this time-point were used to populate the 6 month visit CRF.

Our study therefore achieved a 95% (38 out of 40) overall participant retention rate which compares favourably to reported rates of surgical clinical trials.[198]. This is an important finding in relation to designing the larger definitive RCT.

Figure 2.5: AOT Trial Consort Flow Diagram



2.4.5.4 *Baseline Characteristics*

Baseline demographics are summarized in Table 2.3 showing comparable mean age, gender and ethnicity. As expected and in keeping with previous reports [169, 172], there is a male preponderance.

Table 2.3: Baseline Demographic Characteristics

	Adjunct Group (N=20)	Control Group (N=20)
Number of Patients (Eyes)	20	20
Male/Female	18/2	16/4
Mean Age (yrs)	44	37
Ethnicity:		
White	18	14
Black	1	3
Asian	1	3

Ocular injury types according to the BETTS classification [175] were similar across the two groups, although there were two perforating injuries in the adjunct group, with none in the control group. The incidence of IOFB injury was also higher in the adjunct group. (Table 2.4)

Table 2.4: Ocular Injury Classification

	Adjunct Group (N=20)	Control Group (N=20)
Injury, col(%):		
Rupture	5(25)	7(35)
Penetrating injury	7(35)	12(60)
Perforating Injury	2(10)	0
IOFB	6 (30)	1(3.3)
Location of wound		
Corneal	4(20)	6(30)
Scleral	8(40)	8(40)
Corneal & Scleral	8(40)	6(30)

A relative afferent pupillary defect was present in 8 out of 20 patients (40%) in the adjunct group compared to 9 out of 20 (45%) in the control group. A clear crystalline lens was documented in only 7 patients (3 in the adjunct group and 4 in the control group). Over half of patients (21 of 40) showed signs of lens opacity and one quarter (25%) were rendered aphakic by the initial injury. Lens opacity and aphakia was comparable between the two groups at baseline. Intraocular haemorrhage in the anterior segment was also relatively equally distributed, as a microscopic hyphaema was recorded in 7 out of 10 adjunct patients compared to 10 out of 20 patients in the control group. Severe anterior chamber haemorrhage (>25% hyphaema) was documented at baseline in 3 patients in the adjunct group and 4 in the control group.

Table 2.5: Baseline Ocular Characteristics; anterior segment

	Adjunct (N=20)	**Standard(N=20)**
Laterality (Left eye), col%	7(35)	12(60)
RAPD positive, col%	8(40)	9(45)
VA, median(IQR) ETDRS	0(0-0)	0(0-0)
IOP (mmHg), Median(IQR)	12(8-16)	11(5.5-15)
Refraction, Median(IQR)	0(0-0)	0(0-0)
Hyphaema, col(%)		
None	7(35)	4(20)
Microscopic	7(35)	10(50)
Organised	6(30)	6(30)
Lens Status, col%		
Clear	3(15)	4(20)
Cataract	12(60)	9(45)
Aphakic	5(25)	5(25)
Not possible	0	2(10)

Considering posterior segment characteristics at baseline, vitreous haemorrhage was universally present. This is unsurprising, as our cohort comprises eyes undergoing pars plana vitrectomy following an open globe injury. They are therefore subject to selection bias towards posterior segment pathology (i.e. vitreous haemorrhage or RD) by their inclusion in this study.

Retinal detachment was present in 10 out 20 patients in the adjunct group and 11 out of 20 in the control group (Table 2.6). Duration of RD was also similar between the two groups.

Proliferative vitreoretinopathy (PVR) status was deemed to be more accurately assessed at the time of vitrectomy surgery, rather than at the slit-lamp baseline assessment. An intraoperative assessment is therefore not limited by media opacity and also controls for the potential 2 week time lag between slit-lamp biomicroscopic assessment and operative intervention. PVR of any grade was present at the time of the study vitrectomy in 7 patients in total (2 in the adjunct group and 5 in the control group). The presence of established PVR (Grade C) was present in both patients in the adjunct group and 4 out of the 5 patients in the control group. (Table 2.6)

The median Birmingham Ocular Trauma Score was comparable across both groups and was calculated to be 53 (IQR 37-70) in the control group compared to 49 (IQR 43-70) in the adjunct group.

Table 2.6: Ocular Baseline Characteristics; posterior segment

	Adjunct (N=20)	Standard(N=20)
Vitreous Haemorrhage, col%		
+	2(10)	3(15)
++	12(60)	8(40)
+++	3(15)	3(15)
NP	3(15)	6(30)
Retinal detachment, col%	10(50)	11(55)
Tractional RD, col%	2(10)	2(10)
Duration of RD (median) IQR	15.5(8-21)	10(6-19)
Macula Attached	0	0
PVR Grade:		
B	0	1(5)
C	2(10)	4(20)

2.4.5.5 *Surgical Techniques*

The operative techniques during the primary study vitrectomy were comparable between the two groups (Table 2.7). Silicone oil was chosen as the primary tamponade agent in approximately one half of eyes in both groups. Eight eyes in both groups required surgical induction of a posterior vitreous detachment (PVD).

Table 2.7: Operative Techniques

	Adjunct (n= 20)	**Control (n=19)**
Retinopexy		
Endolaser only	6 (30)	5(26.3)
Cryotherapy	4(20)	2(15.8)
Cryotherapy and Endolaser	7(35)	8 (42.1)
None	3(15)	3(15.8)
Intraocular tamponade		
SF_6	3(15)	3(15.8)
C_3F_8	1(5)	1(5.3)
Oil (1000 cs)	11(55)	7(36.8)
Oil (5000 cs)	0	4(21.1)
None	5(25)	4(21.1)
Additional Procedure		
Lensectomy	10(50)	6(31.6)
Heavy Liquid	9(45)	10(52.6)
Membrane Peel	2(10)	4(21.1)
Drainage Retinotomy	2(10)	1(5.3)
Posterior Vitreous Detachment Induction	8(40)	8(42.1)

SF_6 = Sulphur Hexafluride, C_3F_8 = perfluoropropane , cs = centistoke, PVD = posterior vitreous detachment (*Note that some patients had more than 1 procedure)

2.4.5.6 *Primary Outcome Measure*

As previously mentioned, primary outcome data were available for 39 out of 40 patients as one patient in the control group was lost to follow up after the 4-6 week visit.

There was no observed difference in primary outcome between groups: 50% (n=10 of 20) achieved anatomical success without internal tamponade at 6 months in the adjunct group, compared to 47.4% (n=9 of 19) in the control group, Odds Ratio 1.11, 95% Confidence interval 0.316-3.904. (Table 2.8)

Table 2.8: Primary outcome result

All patients	Adjunct (N=20)	Standard (N=19)	Odds ratio(95%CI) Adjunct compare to standard
Success	10	9	1.11(0.316-3.904)
Failure	10	10	
Excluding early withdrawals			
Success	10	9	0.78(0.208-2.913)
Failure	10	7	

The findings were unaffected by excluding early withdrawals.

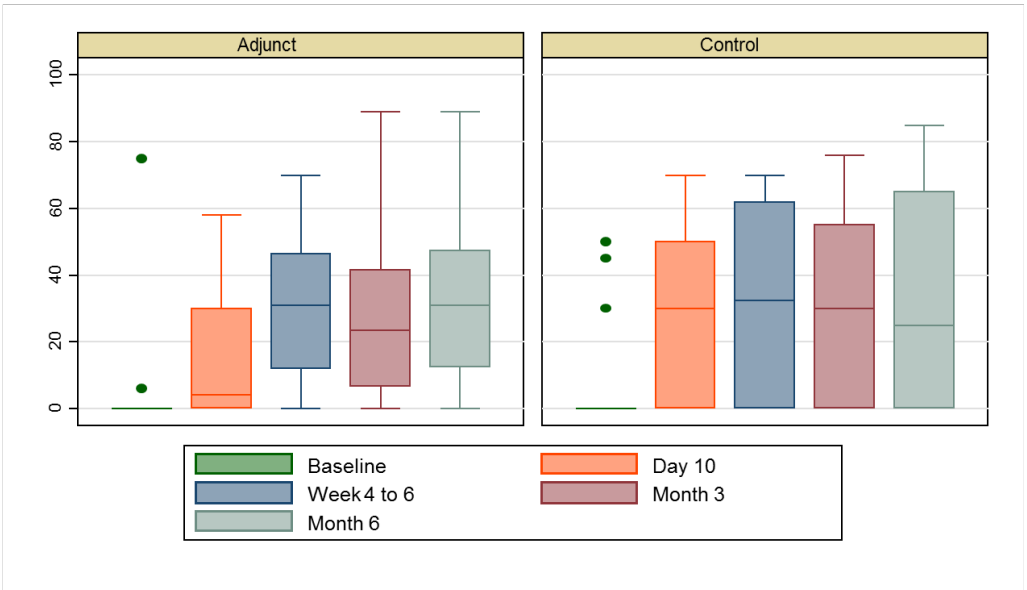
2.4.5.7 *Visual Acuity*

Considering visual acuity at six months following study vitrectomy: final median visual acuity was 31 ETDRS letters (IQR 12.6 - 47.5) in the adjunct group compared to 25 letters (IQR 0-65) in the control group. Three patients (15%) in the adjunct group could read no letters on the ETDRS chart at 6 months compared to eight patients (42.1%) in the control. Five patients in the control group read at least 55 letters compared to three patients who had received the adjunctive regimen (Table 2.9). 16 patients in the adjunct group (80%) gained at 10 letters or more compared to 10 patients (52.6%) in the control group.

Table 2.9: Final Visual Acuity and Change from Baseline

VA at 6 months	**Adjunct(N=20)**	**Control (N=19)**
0 Letters (%)	3(15)	8(42.1)
≥ 55 Letters (%)	3(15)	5(26.3)
VA change from baseline		
Gain 10 Letters (%)	16 (80)	10 (52.6)
Gain 20 Letters (%)	13(65)	10(52.6)
Gain 30 letters (%)	10(50)	8(42.1)
Gain 40 Letters (%)	9(45)	6 (31.6)

Figure 2.6: Box plot of Visual Acuity in ETDRS letters



The visual acuity box plot (Figure 2.6) demonstrates that the median vision in the adjunct group initially lagged behind that in the control group at Day 10 but subsequently exceeded it by month 6. The proportion of patients with visual improvement in terms of number of letters gained is documented in table 4 with a greater proportion gaining 10, 20, 30 and 40 ETDRS letters in the adjunct group compared to the control group. (central bar = median, box = interquartile range, whiskers =range)

2.4.5.8 *Number of operative procedures*

There was no observed difference in the number of operations to achieve success (as defined in the primary outcome measure) with seven patients in each groups achieving success with a single operation, two patients in each group requiring two operations and one patient in the adjunct group undergoing three procedures to achieve stable reattachment at 6 months.

2.4.5.9 *Persistent Subretinal Fluid*

At 6 months, on OCT assessment, only one patient (standard group) was found to have persistent submacular fluid in the presence of an attached retina.

2.4.5.10 *Prevalence of PVR*

Table 2.10 highlights the prevalence of PVR Grade C at each time point, demonstrating a higher prevalence in the control group at day 10, week 4-6 and month 3. At 6 months the prevalence of PVR becomes comparable (35% adjunct groups vs 26.3% control group).

Table 2.10: Prevalence of PVR by time-point

Timepoint	Adjunct(N=20)	Control (N=19)
Baseline	2(10%)	4(21%)
Day 10	0	6(31.6)
4-6 Weeks	2(10)	5(27.8)
Month 3	3(15.8)	6(31.6)
Month 6	7(35)	5(26.3)

2.4.5.11 *Missed Visits*

Each trial patient was scheduled to attend for four postoperative study visits which corresponded to data entry points. There were only seven missed study visits throughout the trial, five of which relate to the two patients who were lost to follow up.

2.4.5.12 *Adverse Events (AE)*

There were a total of 97 adverse events (AE) throughout the study with 59 occurring in the adjunct group compared to 38 in the control group (Table 2.11). However, the proportion of patients suffering at least one AE was 90% (18 out of 20) in the adjunct groups compared to 80% 16 of 20 control patients. There were no serious adverse reactions observed in either group. One serious adverse event occurred in one patient in the adjunct group which was not related to the IMP.

The most frequently occurring AE in both groups was elevated IOP (>25mmHg), with 15 episodes in the adjunct group compared to 11 in the standard group. A slightly higher proportion of patients in the adjunct group (n=7, (35%) suffered at least one episode of elevated IOP compared to five (25%) patients in the control group. Figure 2.7 demonstrates the box plot for IOP comparison by treatment group. There were more cases of postoperative uveitis in the control group (n=5) in comparison to the adjunct group (n=2). An equal number of patients (n=5) suffered at least one episode of hypotony (IOP <6mmHg) during the trial. There were no cases of postoperative endophthalmitis in either group.

There were more single episodes of retinal detachment in the adjunct group compared to the control group (16 vs 8). However, multiple episodes occurred in a small number of patients (4 episodes in one patient and two episodes in two patients) in the adjunct group.

Table 2.11: Adverse Events by Treatment Allocation

	Adjunct (N=20)	Control (N=20)
Raised IOP	15	11
Retinal detachment	16	8
Uveitis	2	5
Further Surgery	15	6
Hypotony	5	2
Other ocular	1	2
Rash/allergy/trauma	3	2
Other Systemic Illness	2	2
Total AE	59	38

Figure 2.7: Box plot of Intraocular Pressure Variation over Time

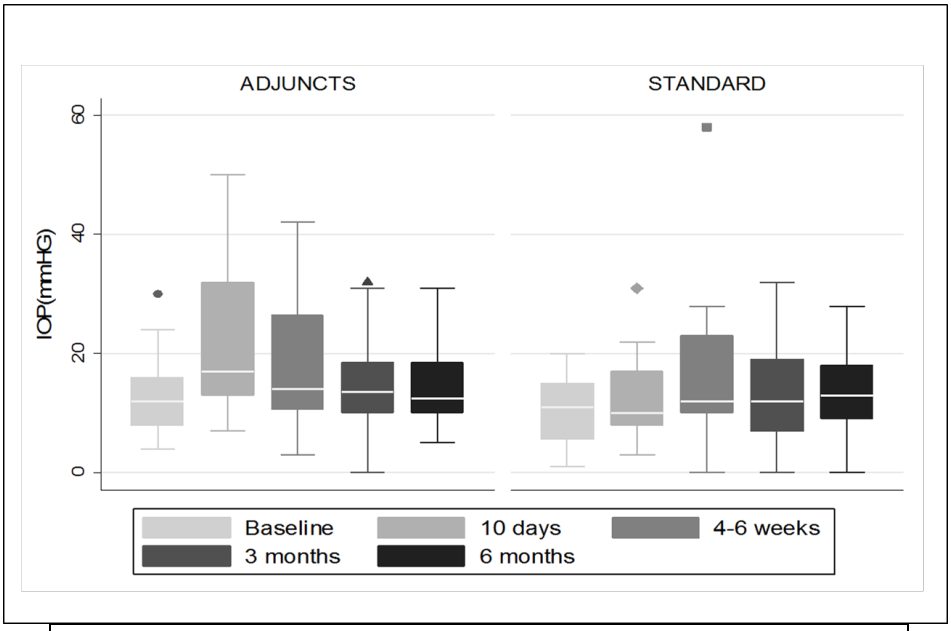


Figure 2.7 demonstrates the median IOP by treatment group over time. The greatest difference was observed at Day 10 post vitrectomy where median IOP was higher in the adjunct group (central white bars = median IOP, boxes = interquartile range, whiskers = range).

2.4.6 Discussion

To date, there have been no prospective randomised controlled clinical trials in this disease group or patient population. The primary aim of this pilot study was to determine the feasibility of the conducting such a trial with a view to provide data to power a definitive RCT.

2.4.6.1 *Recruitment and Retention*

The study was projected to run for 24 months consisting of an 18 month recruitment period and 6 month final patient final follow up. We were able to recruit all 40 participants within 21 months with a favourable eligibility to enrolment ratio of 100%. This in part may be attributable to the pragmatic design of the trial as follow-up schedules and data entry points mirrored standard care. Furthermore, the natural history of both surgical and visual outcome is poor [180] in this disease group, and thus participants were eager to enrol, frequently adopting a 'little to lose' attitude, particularly given the well documented and tolerable side effect profiles of the IMPs. Participant retention rate was also favourable, losing only one patient to follow-up after their 4-6 week visit. This retention rate was higher than expected, with previous surgical trials at the study site reporting a 3-5% loss to follow up rate [11, 97, 98].

2.4.6.2 *Anatomical Outcome*

We observed no difference in anatomical outcome at 6 months with an approximate 50% success rate in both groups. Similarly we did not observe a difference in the number of operations to achieve success. This is the first study to prospectively report anatomical success rates at 6 months in this disease group. Sheng *et* al retrospectively reviewed 90 eyes in elderly patients sustaining OGI [199]. 14 of 15 eyes undergoing secondary PPV with silicone oil for RD achieved anatomical success. However, anatomical success included eyes with an intraocular tamponade *in situ*, and thus it is difficult to draw direct and meaningful comparisons. Taking our cohort

as a whole, we found 19 out of 39 patients (48.7%) undergoing vitrectomy surgery after open globe trauma maintained retinal attachment in the absence of internal tamponade at 6 months, with 14 out of 39 (35.9%) achieving primary anatomical success.

Interestingly, the prevalence of postoperative PVR was higher at each time point up to month three in our control group. This could simply be reflective of a higher incidence of PVR at baseline in the control group (i.e. 5 patients in the control group compared to 2 patients in the adjunct group) , but could also be an indication of a trend towards a treatment effect of the IMP. The prevalence of PVR at month six becomes comparable in both groups. Triamcinolone acetonide when administered as a 4mg/0.1ml Intravitreal injection has been shown to have a duration of effect of between 2 and 4 months[200]. It is possible that the postoperative vitreoretinal scarring response was delayed by the IMP administration, followed by resumption in proliferative activity with falling concentrations of corticosteroid after its clearance.

In a rabbit model of two groups of 42 eyes, Chin *et al* [201] describe a reduced half-life of triamcinolone in eyes which had previously undergone vitrectomy compared to non-vitrectomised eyes. Beer *et al* demonstrated this clinically, and noted increased clearance of a single IVTA injection upon aqueous sampling [196]. Spitzer *et al* describe findings of an *in vitro* study comparing modes of administration of Kenalog in an experimental oil-filled environment [202]. They suggested a depot effect of pre-mixing the corticosteroid with oil prior to injection compared to a single intra-oil administration. They also describe a potential toxic effect in the pre-retinal retro-oil sump of fluid. Due to the pragmatic design of our study we did not elect to premix the triamcinolone preparation. We did not observe any local toxic effects, although no formal electrodiagnostic tests were performed. Furthermore, a collection of corticosteroid in this retro oil fluid may in fact be the preferred distribution of any therapeutic agent targeting the PVR response. Asaria *et al* [203] reported that silicone oil concentrates fibrogenic factors bFGF and Il-6 in this location.

2.4.6.3 *Casemix of Ocular Injuries*

As expected, the nature and severity of the ocular injuries sustained varied markedly within the groups but fortunately not between the two groups. The median Birmingham Ocular Trauma Score [177] was 53 (IQR 37-70) in the control group compared to 49 (IQR 43-70) in the adjunct group. Given the sample size we accept that the sensitivity to detecting small differences between the groups is limited, which may be further confounded by the heterogeneity of the casemix. However, the adequacy of randomisation and comparison of characteristics at baseline showed no significant difference in covariates affecting outcome.

2.4.6.4 *Visual Outcomes*

The visual and anatomical outcome of eyes undergoing vitreoretinal surgery following open globe trauma remains poor. The largest retrospective review to date was by Andreoli *et al.* They reported outcomes of 848 eyes suffering open globe trauma, in which 245 (29%) eyes required vitreoretinal surgery. Despite comparable median baseline visual acuities of hand motions, the reported median final visual acuity was only 20/400 in those eyes requiring vitreoretinal surgery compared to 20/40 in those that did not [180]. In our cohort, the median final visual acuities at 6 months were 31 ETDRS letters (20/250) and 25 ETDRS letters (20/320) in the adjunct and control group, respectively. When considering improvement from baseline as displayed in Table 2.9, where 80% (n=16) gained 10 or more ETDRS letters compared to only 52% (n=10) in the control group, this difference becomes more clear. We also noted fewer patients with very poor visual outcomes (Zero ETDRS letters) in the adjunct group compared to controls (15.0% vs 42.1%). As this is an exploratory pilot study statistical comparisons have not been made, nonetheless the trend is interesting. Covariates which could affect visual outcome such as severity of PVR, number of foveal-off detachments and the use of silicone oil were comparable between the two groups.

Additional clinical factors may affect visual outcome at 6 months. Although the presence of cataract at the final assessment was comparable between both groups, visually significant corneal scarring in those patients with injuries involving zone 1, was not included in the dataset at the final clinical assessment. Its presence in disproportionate numbers in either group could have either exaggerated or mitigated against any observed treatment effect. Likewise, pre-existing ocular comorbidity (e.g. amblyopia or macula pathology) may also affect final visual outcome and should be recorded and adjusted for accordingly in future studies where vision is investigated as the primary outcome measure.

2.4.6.5 *Adverse Events*

As expected, the adverse event (AE) occurring most frequently in the adjunct group was elevated IOP, and accounts for a large number of the total AEs. Interestingly, this was the also the most common AE in the control group as well. A detailed review of corticosteroid related IOP rise will be discussed further in Chapter 3. Absolute AE numbers must be interpreted with caution as this gives limited information on AE occurrence in terms of proportion of patients with a particular AE.

This is important when interpreting the AE data on retinal detachments. P*rima facie* review of the number of RDs may alarm the reader to note twice as many in the adjunct group (n= 16) compared to the control group (n=8). However, repeated events occurring in single patients may explain this disproportionate finding. Furthermore, one patient in the control group was lost at an early stage in the trial (after week four) and one patient did not undergo vitrectomy surgery, thereby reducing the overall 'risk of exposure' to this event.

We did not observe any clear evidence of toxicity of triamcinolone although it is possible that the effects were masked in the context of the complex postoperative appearance in this patient demographic. Concerns regarding the toxicity of IVTA and secondary endophthalmitis have been reported by numerous authors (summarized in [204]) and the preservative benzyl alcohol in Kenalog® has been implicated in its

aetiology. However conclusions have commonly been drawn using higher doses in experimental studies of cultured RPE cells. Oliveria *et al* compared Kenalog® to an unpreserved preparation of triamcinolone in rabbit eyes and found higher mean vitreous concentrations but without evidence of toxicity on electrophysiological and histological analysis [205].

2.4.6.6 *Limitations*

The study is limited as it is single masked and some of the outcome assessments are subjective. However, efforts were made to reduce investigator bias by: 1) masking the operating surgeon to the treatment allocation until the end of the surgical procedure and 2) being explicit in definitions of clinical findings and adverse events and defining rigid management protocols e.g. for IOP rise. An independent assessor of the primary outcome measure at 6 months was considered, but it was felt that there was insufficient evidence to suggest this to be necessary and added additional cost to a pragmatic exploratory pilot study. Furthermore the numbers are small and so estimates of treatment effect may be imprecise.

2.4.7 Summary

This is the first randomised controlled clinical trial in this patient demographic and demonstrates that a prospective randomised controlled clinical trial in this disease group is feasible. Recruitment and retention rates are realistic. The use of intravitreal triamcinolone as a pharmacological adjunct at the time of primary vitrectomy following open globe trauma is safe and suggested a trend towards a better visual outcome in our cohort. An adequately powered definitive RCT is justified and the findings of this pilot study suggests that replacing an anatomical primary outcome in with visual outcome is a potential method of designing vitreoretinal surgical trials.

The results of this study were recently published in the *British Journal of Ophthalmology* [206].

2.4.8 Future and Ongoing work

2.4.8.1 *Public and patient involvement*

INVOLVE is a national advisory group which was first established in 1996 and is funded by the National Institute for Health Research (NIHR). Its role is to support active public involvement in NHS, public health and social care research. It seeks to maximise opportunities for public involvement in research and to ensure that researchers, research commissioners, research funders and the public have access to the support and guidance that they need.

In March 2012, it published a three year strategy plan in which it highlighted four key objectives [4]:

- to lead on public involvement across the National Institute for Health Research by encouraging and facilitating a coordinated approach for promoting and developing involvement in NHS, public health and social care research
- to build and share the evidence base
- to develop capacity and capability for public involvement in research
- to influence research policy and practice

The Adjunct in Ocular Trauma trial met its research objectives. It confirmed that a randomized controlled clinical trial was feasible in patients suffering open globe trauma and it generated a robust outcome data set.

Despite no obvious difference in anatomical outcomes, we observed an interesting trend towards a positive treatment effect in terms of improvement in vision from baseline.

We met with patients who had participated in the trial to involve them in the design of the definitive large scale RCT. We asked whether they felt that vision or anatomical

success should be investigated as the primary outcome. There was unanimous agreement that vision was the more appropriate.

In collaboration with the affiliated Clinical Trials Unit at Kings College London, we were able to secure a research grant from the National Institute of Health Research (NIHR) via the Health Technology Assessment (HTA) funding stream to fund the definitive large scale RCT ; the Adjunctive Steroid Combination in Ocular Trauma (ASCOT) Trial.

2.4.8.2 *PPI in development of ASCOT*

One patient who had exited the study reviewed the study protocol submission and contributed extensively to its design. This patient was a retired physician and clinical trialist whose professional remit included the review of study protocols and his input from both a professional and patient's perspective was valued greatly.

The AOT pilot study had a lay person who sat on the trial steering committee and agreed in principle to sit on the steering committee. She reviewed the participant information leaflet to help ensure its clarity and accessibility to the lay person.

Patient input greatly contributed towards the decision to change the primary outcome measure to a measure of visual acuity, as it was unanimously considered to be most important to them.

Patients were asked to rate the severity of their injury in terms of impact on their life. Five patients attending follow up were asked to rate their eye injury and compare it to either an illness or another bodily injury. All patients rated their eye injury as more severe than a recoverable life threatening illness such as cancer. Three patients equated their injury to loss of a digit or limb, whilst one rated their eye injury to be more severe.

The majority of patients who suffer an open globe injury are healthy males from the working population [169]. The research team asked the patients in the current pilot who were in full time employment to estimate the number of days they have taken off work as a result of their eye injury. We obtained data from 18 of 20 working patients and found that the median length of time away from employment was 60 days with a range between 7 and 330 days.

2.4.8.3 *Lessons learned from pilot and modifications to definitive trial*

As discussed in section 2.4.8.1, a greater emphasis has been placed on PPI during protocol and project development of the ASCOT study. This has resulted in a shift of focus away from investigating anatomical success as a primary outcome, and concentrated on vision. Furthermore, we have collaborated with the Health Economics Department at Bangor University and included quality of life parameters in the secondary outcome measures of the definitive study.

Figure 2.5 (AOT consort) confirms that all participants randomised to receive adjunctive therapy received the primary IMP, triamcinolone, but that flurbiprofen was omitted in 3 out of 20 patients (15%). We therefore agreed to omit flurbiprofen from the adjunctive regimen in the ASCOT study. We have also simplified the pre and postoperative topical regimen to reflect standard care in both groups.

The lessons learned regarding safety reporting are detailed further in Chapter 3 where their relevance sits more naturally in the discussion regarding the competent authority sponsor inspection. Adverse events occurred uniformly across both groups without evidence of serious adverse reaction. We have therefore adopted a risk-adapted approach to the ASCOT study and modified our safety reporting to reflect this. This aims to deliver a pragmatic trial, reflective of clinical practice in a multicentre trial setting. A brief overview of the ASCOT trial is herein described.

2.4.8.4 *Adjunctive Steroid Combination in Ocular Trauma (ASCOT) Trial.*

The ASCOT trial is a phase 3 prospective, multi-centre double-masked randomised controlled trial in eyes undergoing vitreoretinal surgery for open globe trauma. It aims to compare the effect of using adjunctive intraocular and periocular steroid (triamcinolone acetonide) versus standard treatment in this disease group. There are 26 study sites of varying size throughout the U.K. The primary study site is based at Moorfields Eye Hospital NHS Foundation Trust of which I have been appointed as site Principal Investigator (PI).

The trial incorporates a two-stage internal pilot study to verify recruitment and retention rates. In total 300 patients will be recruited and randomly allocated to two treatment arms. Both groups will receive standard surgical treatment and routine preoperative and postoperative treatment and care. The treatment arm will receive additional preoperative steroid combination (triamcinolone acetonide) consisting of 4mg/0.1ml into the vitreous cavity and 40mg/1ml subtenons. The additional IMPS (flurbiprofen and Guttae Prednisolone) included in the AOT trial have been omitted in the ASCOT study. Participants and primary outcome assessors will be masked to treatment allocation. In keeping the pragmatic study design it aims to mirror standard NHS care and thus postoperative examinations will not differ from the schedule followed for vitreoretinal cases and participants will be followed up for 6 months post-surgery. The primary outcome will be corrected visual acuity measured in ETDRS letter score at 6 months. Removal of silicone oil, when used, (combined with cataract extraction + IOL implantation when applicable) will be planned for 3-5 months following study vitrectomy surgery.

2.4.8.4.1 Sample size calculation and primary outcome choice

Aside from study feasibility, a key aim of the AOT trial was to provide data to power the definitive study. I have therefore chosen to include a section on this in this thesis. It must be acknowledged that the calculations were performed primarily by Victoria Cornelius and Jessica Lo from the medical statistics department at Kings CTU. I was, however, involved in the choice of statistical method and acquisition of published data in order to complete the process. A summary is herein included:

Whilst the AOT trial investigated an anatomical primary outcome, we chose to replace this with a primary outcome of visual acuity. As discussed above, this was influenced by patient involvement during protocol development, in addition to directives initiated by the funding body, the NIHR.

Published data indicate that at six months, the distribution of best corrected visual acuity (BCVA) using the ETDRS letter score will be skewed in this patient population [180]. This is in line with results from the AOT pilot which confirms that the majority of participants had a baseline visual acuity of zero (35/40). Additionally, the shape of the distribution of VA at six month differs between the active and control arm. Both of these factors impact the choice of suitable methods for analysis and thus an appropriate approach to calculate the sample size.

The mean difference observed in VA between arms in the AOT study was 3.1 (32.9 – 29.8) with a pooled standard deviation of 28.9. This summary statistic which showed a small average difference between arms may not have fully reflected the true benefit that participants received. Sixteen of 20 patients (80%) versus 11 (55%) gained a meaningful improvement in VA of 10 letters or more in the active versus control group, respectively (Refer to Table 2.9). It was therefore thought to be important to assess both the mean change and the difference in proportion of participants with a clinically meaningful difference in outcome between arms. As a consequence a dual approach to evaluating the primary outcome was taken as recommended by Peacock *et al* [207].

The two cut-offs for statistical significance are each set at 2.5% to provide a test of the composite null hypothesis that the active treatment is no different from control with overall significance of 5% If either the mean difference or the difference in proportions is significant using this cut-off, the composite hypothesis is rejected and it is concluded that the active treatment is superior to control. With 140 per group and using the cut-off for significance of 2.5% we will have 90% power to detect a 20% increase (55% to 75%) in participants who have a meaningful minimum improvement in VA of at least 10. Similarly, with 140 per group and using a 2.5% significance level we would also be able to detect a mean difference of 10 letters with 80% power assuming a standard deviation of 28.

Previous trials run at the primary study site and involving tertiary teaching hospitals maintained a >95% participant retention rate at six month follow up which was confirmed in the AOT trial [11, 97, 98, 206]). As this is a multi-centre trial including non-specialist centres a lower retention rate may be anticipated. Therefore allowing for a 7% dropout rate we will aim to recruit 300 participants to the trial.

A change of 10 letters is widely accepted to be a clinically meaningful in research studies of eye disease [11, 73, 77, 78, 97, 98, 110, 146, 147, 208].

2.4.8.4.2 ASCOT Study Status

At the time of submission of this thesis 21 of 26 sites have gained R and D approval, with 20 sites granted green-light status and open for recruitment. The primary study site at Moorfields Eye Hospital opened for recruitment in December 2014. Projected recruitment rates based on the findings of the AOT trial, estimated recruiting three patients per two month period.

3 Slow-release dexamethasone preparation in the clinical management of eyes with proliferative vitreoretinopathy

3.1 Background

As previously outlined in Chapter 1, proliferative vitreoretinopathy (PVR) is the most common cause of late anatomic failure in retinal detachment surgery and is generally regarded as having an incidence of 5-11% of all rhegmatogenous retinal detachments[12]. PVR may be considered a maladapted wound healing response in specialised tissue. The formation and contraction of fibrocellular membranes on both retinal surfaces and posterior hyaloid face can distort normal retinal architecture with visually detrimental sequelae.

PVR represents a difficult vitreoretinal surgical challenge and despite the improvements instrumentation and technique, surgical failure is common. Multiple procedures are frequently required to eventually achieve final retinal attachment with poor visual results and unsatisfactory binocular visual outcomes [13, 54, 208]. Additionally, PVR management is costly in patient time and healthcare resources [208].

Numerous adjunctive medications have been previously trialled clinically [11, 95, 97, 98, 110, 121, 138, 209], yet no effective and safe adjunct has gained widespread acceptance to improve surgical and visual outcomes. There is a clear need to develop further strategies to improve the outcome in eyes with PVR. A prospective randomised controlled clinical trial investigating the proposed benefit of a novel pharmacological adjunct is justified.

3.1.1 Rationale

The scientific rationale behind the use of corticosteroids in this disease process has already been outlined in Chapter 1. . Both preclinical and clinical evidence have been so far described. Further elaboration will now follow rationalising the choice of pharmacological adjunct which was adopted in this large prospective clinical trial. The duration of action of triamcinolone acetonide may be reduced in vitrectomised eyes, and may offer an explanation as to why it has not emerged as a definitive adjunct. The adoption of a longer acting sustained-release preparation may offer additional advantages.

Dexamethasone has a potency which is five times greater than triamcinolone [210] , and being more hydrophilic, allows for higher vitreous concentrations [211]. However, its clinical utility had previously been limited by its short half-life of three hours [212] and therefore prompted the development of a slow release drug delivery system.

3.1.2 Investigational Medicinal Product

Ozurdex® is a biodegradable intravitreal implant containing 700 micrograms of unpreserved dexamethasone in a slow-release preparation. The implant itself is approximately 6mm in length and 0.46mm in diameter, contained in a disposable injection applicator. The implant is made of a solid biodegradable polymer (Novadur™, Allergan, Irvine, CA, USA). The byproducts of its degradation are glycolic acid and lactic acid, which are subsequently converted to carbon dioxide and water [210]. It has emerged as an alternative therapeutic agent to triamcinolone acetonide and first obtained a market authorization for ophthalmic use in 2010.

Figure 3.1: Slow-release dexamethasone (Ozurdex)

Figure 3.1: Slow-release dexamethasone (Ozurdex) , the rod-shaped implant is supplied preloaded in a disposable injection device (image supplied courtesy of Allergan Inc.

3.1.2.1 *Pharmacokinetics of slow-release dexamethasone implant*

Chang-Lin *et al* [213] described in detail the pharmacokinetic and pharmacodynamic properties of the slow-release dexamethasone implant (Ozurdex®) in an experimental *in vivo* study of 37 male monkeys (*macaca fascicularis)*. Thirty four macaques were injected bilaterally with the 700ug implant and three primates were used as controls. Pharmacokinetic data were determined from plasma, vitreous and retinal samples harvested from day 7 to day 270. Levels of dexamethasone were quantified using liquid chromatography-tandem mass spectrometry. Pharmacodynamics of the released drug were assessed using the expression of dexamethasone-sensitive gene cytochrome P450 3A8 (*CYP3A8*) as a marker of biological activity.

Dexamethasone concentration in the retina and vitreous humor were characterised by two distinct phases which corresponded to the observed fragmentation of the implant. The first phase consisted of initially high drug concentrations observed at day 7 and peaked at day 60 with a C_{max} of 1110 +/- 284 ng/g and 213 +/-49 ng/mL in the retina and vitreous, respectively. There then followed a second phase of sustained lower concentration observed up to day 210 in the retina and day 180 in the vitreous. Thereafter, concentrations of dexamethasone were below the level of quantification (BLQ) in either tissue. Plasma levels were detected up to day 60 (C_{max} = 1.11 +/- 0.11ng/mL) and thereafter were undetectable.

The pharmacodynamic profile mirrored the aforementioned pharmacokinetic properties. CY3A8 expression exhibited a similar dual-phase pattern , with a three-fold increase observed up to month 2 (day 60) followed by a lower but consistently elevated level of gene expression up to month 6 (day 180), which subsequently returned to control levels thereafter.

Chang-Lin *et al* likened this dual-phase pattern to the regimen employed with the administration of systemic corticosteroids i.e. an initial short phase of high corticosteroid concentration after pulsed intravenous methyl prednisolone, followed

by the lower doses achieved with subsequent oral prednisolone. This regimen, has been shown to induce T cell apoptosis [214, 215], and may therefore be advantageous to manage conditions in which T lymphocytes are contributory, as in PVR (refer section 1.3.2)

They went on to compare their findings to previously reported data on alternative modes of dexamethasone administration. They concluded that the slow-release preparation achieved higher and more stable posterior chamber levels of dexamethasone compared to subconjunctival, periocular, topical, or oral administration. (Table 3.1)

Table 3.1: Comparison of vitreous concentration achieved by mode of administration

Mode of Administration	Dose of Dex (mg)	Mean C_{max} (min, max)	Time after last administration (units)
Topical† [216]	0.55	1.1 (BLQ, 1.6)	21-128(minutes)
Oral[217]	7.5	5.2 (1.7, 23.4)	4-10 (hours)
Peribulbar [218]	0.5	13 (4.4, 208)	4-8 (hours)
Subconjunctival [219]	2.5	72.5 (NR)	3 (hours)
Intravitreal [220]	0.4	67.4 (13.9, 392)	60-73 (hours)
Ozurdex	0.7	213 (125, 252)	7-60 (days)

Dex, dexamethasone, BLQ, below the limit of quantitation; NR, not reported. † Cumulative dose of 10–11 drops of 0.1% DEX administered over 15 hours.

They concluded that the controlled release of dexamethasone into the vitreous allowed for lower and less frequent doses to be delivered directly towards the target tissue, thereby avoiding the unwanted side effects of systemic corticosteroid use, or the need for repeated injections.

3.1.2.2 *Licensed indications of Ozurdex ®*

In July 2010, the slow-release dexamethasone implant, Ozurdex, was first granted a market authorization in the U.K for the following indications:

- the treatment of adult patients with macula oedema following retinal vein occlusion (either branch or central) [221]
- the treatment of adult patients with inflammation of the posterior segment of the eye presenting as non-infectious uveitis [222]

Four years later, in July 2014, the European Union's Committee for Medicinal Products for Human Use (CHMP) recommended extending the market authorization for Ozurdex to include:

- the treatment of adult patients with vision loss due to diabetic macular oedema (DMO) who are considered insufficiently responsive to, or unsuitable for non-corticosteroid therapy

A summary of the findings of the three studies which support the current licensed indications of Ozurdex® will follow.

3.1.2.2.1 GENEVA Study

The GENEVA study [221] was a phase III prospective randomized controlled clinical trial of pooled data comparing the visual outcome of patients suffering macular oedema secondary to either branch or central retinal vein occlusion. A total of 1267 patients were allocated to one of three treatment groups; 427 eyes received a single 700ug dexamethasone implant (Ozurdex®), 414 eyes received a 350ug slow release dexamethasone implant and 426 control eyes were administered a sham injection. Approximately two thirds of patients had suffered a branch retinal vein occlusion with the remaining third of patients, eyes with a CRVO. Presenting BCVA was between 20/50 and 20/200 secondary to intraretinal oedema of ≥300 μm in the central 1 mm macular subfield. Macular oedema was required to be present for at least 6 weeks and up to 9 months for CRVO patients and up to 12 months for patients with BRVO. The primary outcome measure was time to achieve a best corrected visual acuity (BCVA) of ≥15 ETDRS letters. The authors reported a higher proportion of patients achieving the primary outcome at all timepoints from day 30 to day 90.

Table 3.2: Summary of Primary Outcome Findings of GENEVA Study

Visit	**OZURDEX®**	**Sham**
	N = 427	**N = 426**
Day 30	21.3 %*	7.5%
Day 60	29.3%*	11.3%
Day 90	21.8%*	13.1%
Day 180	21.5%	17.6%

Proportion of patients (pooled ITT population) with ≥ 15 letters improvement from baseline best corrected visual acuity in the study eye (* denotes significant difference, $p<0.001$)

Additionally, secondary outcome findings reported fewer patients losing ≥ 15 ETDRS letters in the treatment group compared to the sham group. Furthermore, the mean reduction in central macular thickness was 207.9um in the Ozurdex group compared to 95um in patients who received the sham injection a day 90 (p=< 0.001). However, this lost statistical significance by day 180. The second phase of the trial was open-label and eyes were eligible for first or re-treatment. Eligibility required a BCVA of <84 letters or retinal thickness of >250 microns. Those receiving their second implant continued to respond better than those with delayed treatment. The incidence of raised IOP was greater in the Ozurdex group compared to the sham group, with 3.2% of patients recording an IOP of >35mmHg at month 2. Rates of cataract were low, but higher in the Ozurdex (7%) compared to 4% in the sham group. A detailed discussion regarding raised IOP and cataract will follow later in this chapter in section 3.2.7.6.

3.1.2.2.2 HURON Study

The HURON study provided the clinical evidence leading to the market authorization of Ozurdex in the treatment of non-infectious intermediate and posterior uveitis [222]. This was a phase 3, multicentre, randomized controlled clinical trial of 229 patients divided into three equal groups. Eyes received either the 700ug Ozurdex implant, 350ug implant or sham injection. The primary outcome was the proportion of eyes with a vitreous haze score of zero at week 8. Secondary outcome measures included time to vitreous haze resolution, reduction in haze and BCVA improvement. The proportion of eyes treated with Ozurdex achieving the primary outcome (47%) was significantly greater than with sham (12%). The effect was maintained up to week 26. Secondary outcome measures (BCVA and vitreous haze improvement, reduction in rescue treatment) showed similar treatment effects.

Elevated IOP (>25mmHg) was observed in 19 of 76 (25%) patients who received Ozurdex compared to 5 patients (6.7%) in the sham group.

3.1.2.2.3 The MEAD study

The MEAD study [223] reported findings on the efficacy and safety of Ozurdex® in eyes with DMO. It comprised two parallel randomized, multi-centre, masked phase III clinical trials of 1048 patients with DMO. A BCVA of 20/50 to 20/200 and OCT-measured CMT of ⩾300 μm was a requirement for trial entry. The patients were randomized in a 1:1:1 treatment allocation ration as in the GENEVA and HURON trials and prospectively followed for 3 years. Re-treatments were restricted to 6 monthly intervals. The primary outcome measure was the proportion of eyes gaining 15 ETDRS letters from baseline to study exit. Secondary outcome measures included a comparison in mean reduction in central foveal thickness (CFT) from baseline.

A significantly higher proportion of eyes in the Ozurdex® group (22.2%) achieved the primary outcome compared to 12 % in the sham group (p = 0.018). The mean reduction in CFT from baseline, was also greater in the treatment groups (−111.6 μm) compared to *sham* (−41.9 μm; $p < 0.001$). However, treatment efficacy in the steroid group appeared to be lost prior to the 6 month retreatment interval. Furthermore, study retention was poor, with over one third of patients in the steroid treatment groups and over half in the sham group exiting the study early [224].

3.1.3 Slow-release dexamethasone (Ozurdex®) in vitrectomised eyes

The three aforementioned studies provide evidence to support the use of the slow release dexamethasone implant for its licensed indications. However, no comment has been made regarding its use in eyes which have previously undergone vitrectomy. A review of pre-clinical and clinical evidence covering this topic will follow.

3.1.3.1 *Preclinical Evidence*

The clinical effectiveness of using pharmacological agents in eyes which have previously undergone vitrectomy surgery has been questioned as the pharmacokinetics may be altered in this environment. Increased drug clearance in vitrectomised eyes injected with anti-vascular endothelial growth factors (anti-VEGF) [225], triamcinolone [201, 226], 5' FU [227] and amphotericin[228] have all been previously reported.

Chang-lin *et al* published a second report in 2010 comparing the pharmacokinetics of the Ozurdex® implant in vitrectomised and non-vitrectomised experimental eyes [229]. Twenty five rabbits underwent vitrectomy surgery in one eye, with the fellow non-vitrectomised eye serving as a control. An implant was injected bilaterally and the drug release profile of dexamethasone was measured in the vitreous humor and retina, at weekly intervals from day 2 to 31 using their previously described methods [213]. They found no significant difference in the concentrations of dexamethasone in either tissue at any timepoint ($p > 0.05$). The maximum concentration of dexamethasone in non-vitrectomised versus vitrectomised eyes was 4110 ng/mL at day 15 compared to 3670 ng/mL at day 22 in the retina, respectively. For vitreous humor, the maximum concentration was 791ng/mL versus 731 ng/mL at day 22. They concluded that the pharmacokinetic profile of Ozurdex® was similar in the two posterior chamber environments.

3.1.3.2 *Clinical Evidence*

The CHAMPLAIN Study was a multicentre, prospective, non-controlled, open-label study investigating the safety and efficacy of Ozurdex® in vitrectomised eyes with DMO [230]. Patients with a central retinal thickness ≥ 275 µm on OCT, and BCVA from 24 letters to 70 letters were eligible. Fifty five of 56 patients received a single treatment of Ozurdex at baseline and were followed for 6 months. Primary outcome measure was mean reduction in central macular thickness (CMT) at 6 months. Secondary outcomes included BCVA improvement and safety data.

A significant reduction in CMT was noted at week 8 (-155.9µm) and persisted to month 6 (-39 µm). A corresponding improvement in vision of 6 and 3 letters was noted at both timepoints, respectively.

At least one episode of raised IOP (>25mmHg) was noted in 16% of patients, the prevalence peaking at week 8 and reducing to zero upon study completion. Cataract progression was observed in 17% (2 of 12) phakic patients. The authors concluded that treatment with the slow-release dexamethasone implant in this population led a statistically and clinically significant improvement in DMO and vision, with an acceptable safety profile.

Furthermore, other authors have reported on vitrectomised eyes where the slow-release preparation has successfully treated refractory macular oedema secondary to uveitis, vascular occlusions, and post vitrectomy for retained lens fragments [231-233].

3.1.4 Ozurdex® and Silicone Oil

The pharmacokinetics of Ozurdex® has been described, exhibiting a dual-phase response of initially high concentrations of dexamethasone in the first two months, followed by a period of lower concentrations sustained for up to 6 months post injection [213]. Furthermore, vitrectomy has not been shown to significantly affect the drug release profile [229]. Nevertheless, the proposed clinical trial in which Ozurdex is to be used as pharmacological adjunct, eyes have not only undergone vitrectomy surgery, but will also be filled with an intraocular tamponade of silicone oil.

As there is no published data on using Ozurdex® in this intraocular environment, the manufacturing company (Allergan, Inc.) were contacted to provide further information. An overview of unpublished data of an *in vitro* study conducted by Allergan is herein described [234].

The study investigated the drug-release profile of dexamethasone from the Ozurdex implant in a saline/silicone oil medium and compared it to a control. Three experimental groups were investigated as follows: the first two groups each had six replicates of a forty milliliter (ml) medium containing 30 ml of 0.9% saline and 10ml silicone oil, the third group (control) consisted of a saline only medium, although the volume was not reported. Groups 1 and 2 differed in the manner in which the implant was introduced to the medium. In group 1, termed DSO, the implant was added to the glass vial prior to adding the saline and then silicone oil media. In group 2, termed SOD, the sequence of vial fill was saline, followed by silicone oil, followed by the 'placing' of the Ozurdex implant over the oil layer. Dexamethasone concentrations were measured in both the saline layer and the silicone oil layer and sampling was performed weekly for 28 days.

The calculation to estimate the percentage of dexamethasone released was as follows:

$$\% \text{ Dex} = \frac{\text{Concentration of dexamethasone released (µg/ml)x30 ml saline}}{\text{Calculated amount of dexamethasone in Ozurdex}} \times 100\%$$

Figure 3.2: Drug release profile of dexamethasone in different media

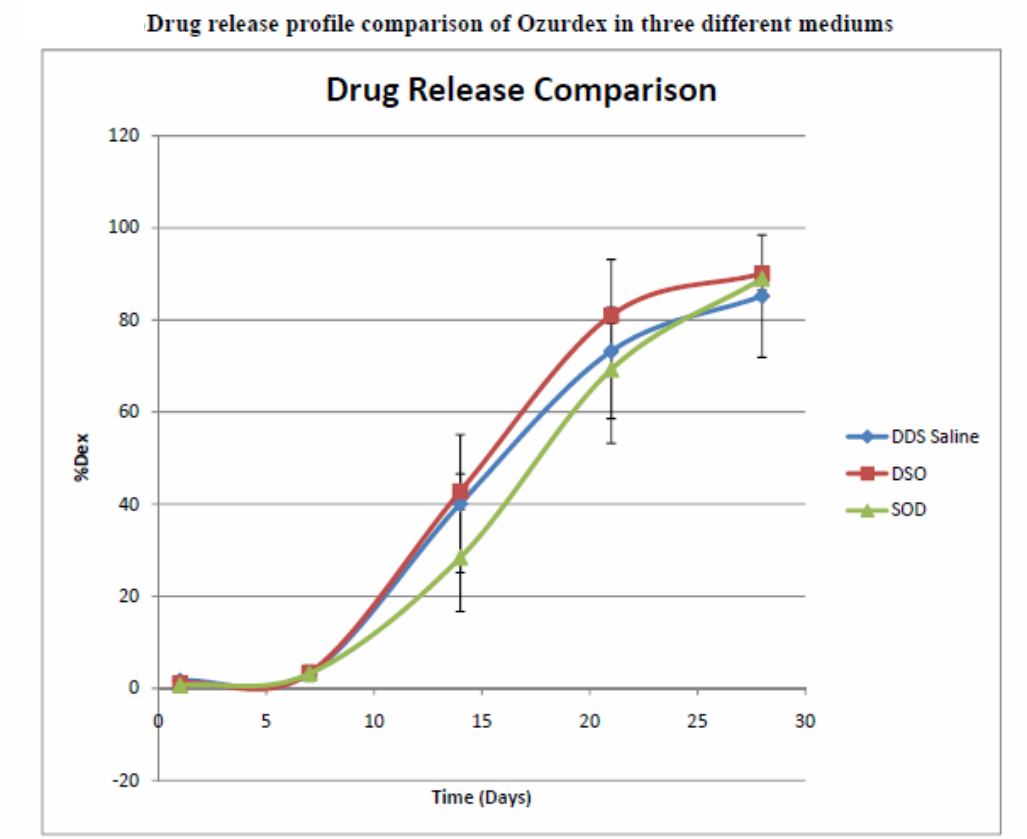


Figure 3.2 (DDS = saline only, DSO = implant, then saline then oil, SOD = saline, then oil, 'placed' implant). Line graph shows that the average percentage of dexamethasone released in saline for all three groups did not differ significantly. In group 2 (SOD) the drug release in saline from Day 1 to Day 21 is slower compared to control and group 1, however, by day 28 the drug release is comparable. (Figure is reproduced unaltered directly from the correspondence provided by Allergan, U.K with permission requested [234])

The investigators concluded that the *in vitro* release profiles of dexamethasone in saline in the presence of silicone oil (groups 1 and 2) versus the saline alone (control) are similar.

Additionally, the concentration of dexamethasone release from Ozurdex in the silicone oil layer was calculated. The silicone oil reportedly used in these experiments was a polydimethyl siloxane and has only trace solubility of dexamethasone (0.90 µg/mL). Dexamethasone concentrations were measured in the silicone oil layer extracted from both groups 1 and 2 on day 28 and showed only trace amounts (<2.5ug/mL) for all samples tested. This is as expected given the lipophobic properties of dexamethasone.

Caution must be exercised when extrapolating this *in vitro* data to *in vivo*. Firstly, the data is unpublished and the experimental methodology is incompletely described. Secondly, the proportion of silicone oil/saline medium does not accurately reflect that of clinical practice. In fact, upon completion of vitrectomy surgery and vitreous cavity air or fluid oil exchange, the surgeon aims to fill the cavity with silicone oil as completely as possible. The resultant oil/aqueous ratio is therefore more usually 90:10, respectively, which starkly contrasts the 10:30 ratio which was investigated *in vitro.*

3.2 A slow- release dexamethasone implant in proliferative vitreoretinopathy; a prospective randomised controlled clinical trial

3.2.1 Purpose

The aim of this study was to determine whether an adjunctive slow release dexamethasone implant given at the time of vitrectomy surgery and repeated at oil removal could improve the anatomical and visual outcomes in eyes with established proliferative vitreoretinopathy

3.2.2 Primary Objective

To test the hypothesis that adjunctive Ozurdex®, given at the time of surgery, can improve the anatomical outcome of vitreoretinal surgery for established PVR i.e. stable retinal reattachment with removal of silicone oil without additional vitreoretinal surgical intervention at 6 months

3.2.3 Secondary Objectives

To determine whether adjunctive Ozurdex®, given at the time of surgery has an effect on the following at 6 and 12 months following primary study vitrectomy:

i) visual acuity (median and ETDRS of 55 letters or better)

ii) macula oedema and thickness (SD - OCT analysis)

iii) development of overt PVR recurrence at any timepoint

iv) complete retinal reattachment

v) posterior (post equatorial) retinal reattachment

vi) tractional retinal detachment

vii) hypotony/raised IOP

viii) macula pucker/epiretinal membrane

ix) cataract

x) Quality of Life

3.2.4 Methods/design

This was a phase III participant masked randomised control study involving 140 patients undergoing vitrectomy surgery with silicone oil for a retinal detachment with established PVR. Participants were randomised into two equal groups (adjunct and control arm) following satisfaction of the inclusion/exclusion criteria. Both groups received the standard surgical treatment appropriate for their eye condition and routine preoperative and postoperative treatment and care, differing only in the addition of supplementary Ozurdex® in the adjunct group. The study protocol has been published in *Trials*, an open access journal [235].

3.2.4.1 *Study Set up*

3.2.4.1.1 Funding

An unrestricted grant was obtained from Allergan, Ireland by Professor Charteris . The funding agency had no role in the protocol design, management or results dissemination of the trial.

3.2.4.1.2 Regulatory authority approval

The process of gaining all regulatory authority approvals was led by me. Moorfields Research Management Committee (RMC) approval was obtained as sponsor of the trial. A favourable opinion from the National Research and Ethics Service Committee London - Central was received (11/LO/1685) and the study was granted a clinical trials authorisation by the MHRA. The trial was registered on the European Clinical Trials Database (EudraCT No 2011-004498-96).

The study was conducted in accordance with the International Conference on Harmonisation for Good Clinical Practice, as set out in the European Union Clinical Trials Directive (2001) and associated UK Regulations (2004). The study complied at all times with the Declaration of Helsinki (2000). Patients provided written informed consent before entering the trial. An independent Data Monitoring Committee (DMC) and Trial Steering Committee (TSC) provided study oversight throughout the duration of the trial.

3.2.4.1.3 Case Report Form Design

A project data guide was provided by the research department applications team at Moorfields Eye Hospital (MEH) in order to develop a case report form (CRF) which adhered to the sponsor's standard operating procedures. Paper case report forms (CRF) were developed by me to ensure the efficient capture of the required dataset (Appendix 4). The study database was designed and programmed by the applications team at MEH. Prior to green light approval, dummy data were entered into the study database in order to test its design.

3.2.4.2 *Eligibility of Participants*

3.2.4.2.1 Inclusion Criteria

Patients with established PVR (grade C) [16] following rhegmatogenous retinal detachment requiring surgery with planned silicone oil tamponade.

3.2.4.2.2 Exclusion Criteria

1. Individuals less than 18 years old
2. History of Open Globe Injury
3. A diagnosis of ocular hypertension on 2 or more pressure lowering medications or a definite diagnosis of glaucoma if in the opinion of a glaucoma specialist, the patient is at high risk of visual damage from raised IOP
4. Uncontrolled uveitis
5. Previous steroid induced glaucoma
6. Proliferative Diabetic Retinopathy or vasculopathy
7. Pregnant or Breastfeeding females (Females of child bearing potential must have had a negative pregnancy test within 7 days of commencing the trial and agree to adequate contraception throughout the duration of the trial)
8. Previous known adverse reaction to the IMP
9. Suspected ocular/periocular infection (e.g. Herpes Simplex Virus, Varicella Zoster Virus, mycobacterial, fungal disease)
10. Aphakia or patients in whom a lensectomy is planned at time of surgery
11. Pre-existing Anterior Chamber Intraocular Lens
12. Inability to give informed consent
13. Unwilling to accept randomization and attend follow-up

There were no restrictions on the number of previous vitreoretinal procedures.

3.2.4.3 *Study Interventions*

Both groups underwent the standard intraoperative procedure appropriate for their clinical condition. Consultants or senior fellows (2nd year fellowship) performed the operative procedures.

This involved a standard 3 port pars plana vitrectomy (or gel trimming if the eye was previously vitrectomised) and internal identification of retinal breaks; peeling of anterior and posterior epiretinal membranes and removal of subretinal membrane was performed as required; relief of traction by retinectomy or placement of a scleral buckle was performed at the operating surgeons discretion; retinopexy to retinal breaks and retinectomy edge by cryotherapy or laser; intraocular tamponade using 1300 or 5000 centistoke silicone oil.

Removal of silicone oil (combined with cataract extraction + IOL implantation when applicable) was planned for four to five months after the study vitrectomy. 360 degree prophylactic barrier laser was performed prior to oil removal at the treating clinicians' discretion.

3.2.4.3.1 Adjunct group

Upon confirmation of successful retinal reattachment and completion of silicone oil exchange, the operating surgeon was asked to clinically grade the level of PVR using the standardized classification system in current practice [16]. Thereafter, the surgeon was asked to inject a 0.7mg slow release dexamethasone implant through the final open sclerotomy port prior to suturing (Figure 3.3).

Slit-lamp and/or indirect biomicroscopy was performed the following day to confirm the position of the implant.

Figure 3.3: Intra-operative Injection of Steroid Implant

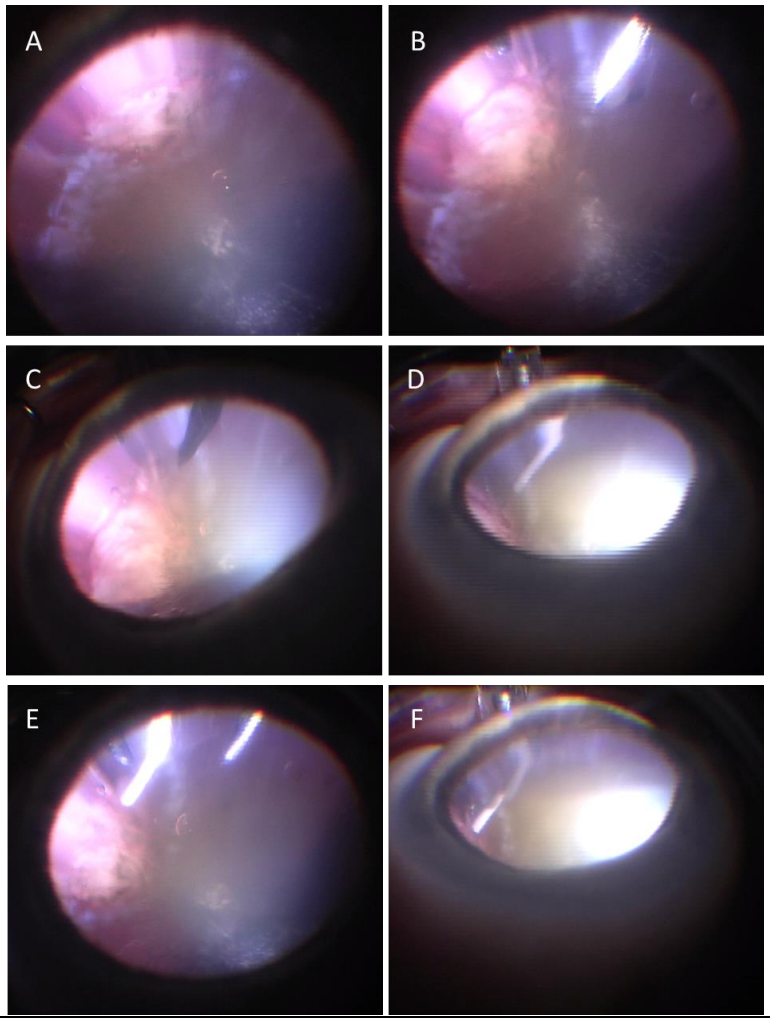


Figure 3.3 Sequence of still photographs highlighting injection of slow-release dexamethasone implant A) retina attached and eye is oil filled, note multiple white blanches of the retina where the retinectomy edge has undergone laser retinopexy, B) bevel of preloaded injector is introduced through sclerotomy and is visible in the operating field as C) bevel is directed towards bare RPE, anterior to retinectomy edge D) the release button on body of applicator is depressed and rod-shaped implant is visible emerging from tip of injector, E) implant travels within oil bubble until impedance and begins to sink deeper into the eye F) injector is removed and implant sinks towards retina to rest on retinal surface

A similar procedure was followed for the second implant administration at the time of oil removal. Upon confirmation that the retina remained attached following removal of oil, the surgeon was again asked to confirm the retinal status and the presence or absence of PVR. As a variety of techniques were used to remove silicone oil, particularly if combined cataract surgery was performed, the implant was either injected through a sclerotomy port (if used) or via the conventional method of delivery [236].

3.2.4.3.2 Control Group

Following successful retinal reattachment, completion of silicone oil exchange and grading the level of PVR, the surgeon was informed that no adjunctive medication was required and the final sclerotomy port was sutured.

3.2.4.4 *Modifications to Study Intervention*

If a patient was rendered aphakic as part of the operative procedure and had been randomised to receive the IMP, the study treatment was not given, but the patient still followed up as part of the study. Reports of migration of the implant into the anterior chamber in unicameral eyes, with secondary corneal decompensation necessitated this safety precaution [237, 238].

Additionally, restrictions were placed on proceeding with the second implant injection depending on preoperative IOP. This is detailed in section 3.2.4.6 in the algorithm for managing IOP.

3.2.4.5 *Schedule of assessments*

3.2.4.5.1 Informed Consent

Informed consent was taken by me or the chief investigator prior to performing any study-specific interventions and after administration of the PIL. Occasionally, emergency surgery was required necessitating trial enrolment within a 24 hour period. In these circumstances, we ensured that patients had been provided adequate time to reach their decision. This was documented in the source documents (hospital clinical notes).

3.2.4.5.2 Screening

A screening assessment was performed to confirm that eligibility criteria were met prior to enrolment in the trial.

3.2.4.5.3 Baseline Assessment

Baseline assessments were performed within one week of the scheduled study vitrectomy date. This included: patient demographics, past ocular history, logMAR visual acuity (ETDRS method), slit lamp/indirect ophthalmic examination (anterior and posterior segment assessment, lens status, extent of retinal detachment, grade of PVR [16]) , spectral domain OCT-guided foveal thickness , and quality of life questionnaires.

3.2.4.5.4 Follow up assessments

Postoperative study visits did not differ from the routine schedule for vitreoretinal procedures at the study site for the first 6 months i.e. day 1, day 10, week 4, 3 months and 6 months. Further assessments were scheduled for 9 and 12 months post initial surgery. The time window allowed around these scheduled visits were as follows: Day 10 (+/- 3 days), Day 30 (+/- 7 days) Months 3, 6, 9 and 12 (+/- 14 days). At each scheduled postoperative study visit, a full ophthalmic assessment was

completed which included slit lamp biomicroscopy (+/- indirect binocular ophthalmoscopy when required) and parameters including ETDRS visual acuity, Goldman applanation tonometry, anterior segment assessment and retinal attachment status were recorded.

3.2.4.5.5 Visual Acuity Assessment

As discussed, visual acuity was measured at baseline and subsequent study visits using the standardised ETDRS method by Margaret Zvobgo (Research Sister) or a delegated substitute. In our study, this consisted of a best spherical corrected vision where patients were assessed reading letters on an ETDRS Letter Chart at four and/or one metre to obtain an ETDRS letter score.

More specifically, If 20 or more letters are read correctly at 4 metres, then the visual acuity score is equal to the number of letters read correctly (N) + 30. If one or more but fewer than 20 letters were read at 4 metres, then the visual acuity score is equal to the number of letters read correctly at 4 metres plus the number of letters read correctly at one metre in the first six lines. If no letters were correctly read, the ability to count fingers (CF), identify hand movements (HM) or perceive light (PL) was assessed.

3.2.4.5.6 Optical Coherence Tomography

Spectral-domain optical coherence tomography was performed by delegated was used to record central foveal thickness adopting a trial- specific scanning sequence as follows:

The Heidelberg Spectralis HRA-OCT Model was used in all patients.

The resolution mode was set to high speed, with a central internal fixation target and the enhanced depth imaging mode (EDI) switched off.

A sequence of 25 horizontal sections that covered an area 20^{o} X 20^{o} (5.6x 5.6mm) was recorded with a distance of approximately 200µm between each individual section. Ten frames (ART 10) were acquired for each B scan location to reduce noise and improve quality. The final OCT image dimensions were 512 X 496 pixels.

Imaging technicians, trained on the scanning protocol acquired the OCT scans. Quantitative measurements were obtained using automated algorithms incorporated into the Heidelberg Software. The presence or absence of cystoid macular oedema and macular pucker were OCT-derived dichotomous variables. Where OCT scanning was not possible (e.g. due to media opacity) a clinical judgement was made.

3.2.4.5.7 Additional Visits and Reoperations

An additional study visit to assess the IOP was included into the protocol schedule at day 60 postoperatively following both the study vitrectomy and the removal of oil procedure. ETDRS visual acuity and applanation tonometry was recorded and entered in to the CRF for data capture.

All reoperations were recorded as adverse events and again study specific CRFs were completed for data capture. Silicone oil removal was not considered a reoperation and routine subsequent follow up ensued until the patient returned to the study visit schedule. Other vitreoretinal interventions (with the exception of isolated retinal laser and macula epiretinal membrane peel) over the trial period were considered reoperations and recorded as such.

Postoperative visits related to reoperations, or other attendances outside the study visit schedule, were recorded as 'unscheduled visits'. CRFs identical in composition to the study scheduled visit CRF were completed and included in the data analysis on completion of the study.

Following the final study visit at 12 months, participants were discharged back to the care of their General Practitioner. Participants requiring ongoing ophthalmic care continued follow up under their admitting consultant or under the care of a more appropriate specialist consultant as required.

3.2.4.6 *Management of intraocular pressure (IOP)*

Management of raised IOP adhered to the following explicit protocol, which was approved by an external glaucoma specialist and is summarised in below:

Table 3.3: Management of elevated intraocular pressure (IOP)

Intraocular Pressure (IOP) (mmHg)	**Treatment**	**Follow up**
≤ 25	None	As per protocol schedule
>25 but <30	Single Topical Ocular Hypotensive	Within 6 weeks *
≥30 but <35	Dual Topical Ocular Hypotensive	Within 6 weeks
≥35	PO Acetazolamide 500mg and dual therapy given Stat (Recheck IOP within 2 hours)	After 2 hours: 1. IOP <35 – PO Diamox 250mg SR bd 5 days + dual topical therapy F/U 1 week 2. IOP ≥ 35 – same day glaucoma service input and/or consultant VR input

* If IOP has not responded to single therapy or only partially responded, then a substitute agent will be tried, or an additional agent added, respectively.

Patients were referred to the glaucoma service if:

1. IOP remained >25mmHg on dual therapy
2. Long term IOP management was required (i.e. >2 consecutive months of IOP lowering agents required in the absence of an internal tamponade agent)
3. the investigators deemed it in the patient's interest

In the event where a patient's IOP was >25mmHg at the time of listing for removal of oil procedure then additional topical ocular hypotensive agents was started/or added and their surgery postponed by up to 4 weeks until the IOP is ≤ 25mmHg. Where the IOP remained >25mmHg or if systemic ocular hypotensive agents was required to control the IOP, then patients in the adjunct group had their second injection omitted. This was recorded and will be discussed in the results.

3.2.4.7 *Recruitment*

Recruitment into the trial commenced following documented REC, Regulatory and Local Trust R&D approval. All 140 participants were identified and recruited from outpatients and emergency referrals at Moorfields Eye Hospital. The study research nurse (MZ) frequently identified individuals with potential eligibility but study enrolment and informed consent was performed by me in approximately 95% of cases.

3.2.4.8 *Randomisation*

After informed consent, patients were allocated to either the treatment arm or control arm. The randomisation list was generated using permuted blocks of varying sizes and was generated by a senior data manager independent to the trial team. The list of 140 study IDs was held by the trials pharmacist, and following recruitment into the trial, participants were allocated to the lowest unused study ID. Out of hours (i.e. weekends and bank holidays), the next study ID in sequence was kept in a sealed envelope in a secure location on site when access to the trials pharmacist was limited.

3.2.4.9 *Masking*

Participants were masked to their treatment allocation until their completion of the study and confirmation of masking status was assessed after the study vitrectomy, at month 6 and upon trial exit. The operating surgeon was masked until the end of the procedure just prior to sclerotomy closure, to avoid any bias regarding surgical management. It was not possible to actively mask the investigators as the IMP was visible on posterior chamber assessment until its degradation. A placebo vehicle was not used as a comparator as it was deemed unethical due to lack of safety data, and scientifically justified by comparing the treatment group to standard care.

3.2.4.10 *Outcome Measures*

3.2.4.10.1 Primary

Stable anatomic reapposition of the retina to the retinal pigment epithelium in the absence of an internal tamponade agent at 6 months post study vitrectomy without additional vitreoretinal intervention

3.2.4.10.2 Secondary

Secondary outcomes were assessed at 6 and 12 months following primary study vitrectomy:

i) visual acuity (a comparison of the median visual acuity and the proportion of patients in each group achieving a VA of 55 ETDRS letters or better)

ii) macula oedema and thickness (OCT analysis) i.e. the proportion of patients in each group with a central A1 macula subfield measure of >300um

iii) the proportion of patients in each group who develop overt PVR recurrence

iv) the proportion of patients in each group achieving complete retinal reattachment

v) the proportion of patients in each group achieving stable posterior (post equatorial) retinal reattachment

vi) the proportion of patients in each group with a tractional retinal detachment

vii) the proportion of patients in each group who suffer hypotony (defined as IOP <6mmHg and/or raised IOP (defined as >25mmHg) at any time point during the study period

viii) the proportion of patients in each group who develop the presence of macula pucker/epiretinal membrane and/or require macula ERM surgery at any time point during the study

ix) the proportion of patients in each group who require cataract surgery at any time point during the study

x) Quality of Life assessment – a comparison in the median/mean scores of both SF36 and VFQ between both groups and the proportion of patients with severe depression

3.2.4.11 *Adverse Events and Safety Reporting*

Safety reporting adhered to the sponsor's standard operating procedures and measures were in place to monitor, record and report adverse events (AE) in line with the MHRA guidelines. AEs were assessed for severity, causality, expectedness and seriousness. An external Data Monitoring Committee was established with an agreed charter to which to adhere. They met six to twelve monthly or on an *ad hoc* basis as required.

For the purposes of this trial we determined the following adverse events as expected occurrences: a) cataract, b) raised IOP, c) hypotony, d) sterile hypopyon, e) retinal detachment, f) uveitis, g) further surgery, h) glaucoma. i) headache, j) migraine, k) vitreous opacities, l) tractional maculopathy

More specifically, the recording of severity of Raised IOP was as follows:

Mild: > 25mmHg <35mmHg; Moderate: ≥ 35mmHg; severe: any interventional invasive procedure (e.g. surgery/laser) required to control the elevated IOP acutely or long term, during the study period

Unexpected adverse events included: a)endophthalmitis, b)systemic illness c)ocular vascular occlusion d) other

3.2.4.12 *Trial Size and time-scale*

The sample size calculation was performed by the study site senior medical statistician as follows:

based on the results of the primary outcome measure from a trial of the same patient group carried out in the study centre [97] 66 patients per study arm are required for a study power of 85% to detect, at the 5% level, a 50% improvement in success of the adjunctive regimen (reducing failure from 49% to 24%). A 50% reduction in failure would represent a marked clinical benefit and would be likely to be adopted on a large scale by vitreoretinal surgeons. Given a 5% loss to follow up and protocol violations (previous similar studies at Moorfields had rates of 3-5% loss to follow up /protocol violation [11, 97, 98], this gives a study total of 140 patients to be randomised.

The trial was funded for 36 months allowing a 24 month recruitment period and 12 month follow up. The projected recruitment rate was therefore 5.83 patients per month.

3.2.4.13 *Data entry*

Greater than 95% of all paper CRFs were completed by me as the primary clinical assessor throughout the trial duration. The remainder were completed by the chief investigator or research nurse. The accuracy of source document reflection was checked by the study research nurse prior to data entry. A second check was performed at the final visit of each patient by both me and the research nurse. A delegated authorised individual (research nurse) entered the data onto the trial database created by the R&D applications team. Data entry was carried out within 1 week of CRF completion.

When all patients had completed their follow-up, an R&D data officer performed double data entry of a random sample of all study data of 10% of the sample size.

Additionally, double data entry was performed for 100% of primary outcome data of all patients in the study.

The first and second data entries were compared for completion and consistency checks were performed according to the sponsor's SOPs. The error rate was calculated and errors were corrected as necessary with completion of investigator site file notes where appropriate.

3.2.4.14 *Statistical analyses*

3.2.4.14.1 Principle

All statistical methods presented in this thesis were performed by me using IBM SPSS Version 22.0. With approval from the internal data monitoring committee, the sponsor formally granted release of the locked data set for my personal use in this thesis. Results of the primary outcome and 6 month secondary analysis are herein included.

As this study was a clinical trial of investigational medicinal product (CTIMP), a formal statistical analysis plan (SAP) was written in advance of the data analysis by the trial statistician (Ms Ana Qartilho, supervised by Dr Catey Bunce). The SAP was reviewed and approved by both myself and the chief investigator and subsequently the Trial Steering Committee. For the purposes of the primary research paper and study report data, concurrent analysis was performed by the trial statistician. The findings of the primary outcome measure analysis were compared to my findings and were identical, thus verifying my statistical methods.

The outcome analysis was by intention to treat (ITT) in order to retain the validity of the randomisation process. The trial ITT population comprised all randomised patients regardless of eligibility (inclusion/exclusion) error, post-randomisation

withdrawal and whether the correct study treatments were received, or other interventions received. All statistical tests use a 2-sided p-value of <0.05 for significance, unless otherwise specified. All confidence intervals presented are 95% and two-sided.

A CONSORT flow diagram was constructed in accordance with 2010 CONSORT Guidelines to report recruitment, randomisation and follow-up summarised by treatment arm.

Baseline characteristics of the two groups were compared to assess the adequacy of randomization. Summary measures of each group are presented as mean and standard deviation (SD) for continuous (approximately) normally distributed variables, medians and interquartile ranges for non-normally distributed variables, and frequencies and percentages for categorical variables.

3.2.4.14.2 Primary Endpoint Analysis

The proportion of patients satisfying the primary outcome (section 3.2.4.10.1) was reported by treatment group with 95% confidence intervals computed by the exact binomial method. A binary logistic regression model (including terms for baseline PVR grade) was used to estimate the treatment effect as an odds ratio reported with 95 % confidence intervals.

3.2.4.14.3 Secondary Endpoint analysis

Summary statistics of each group are presented as mean and standard deviation (SD) for continuous (approximately) normally distributed variables, medians and interquartile ranges for non-normally distributed variables, and frequencies and percentages for categorical variables. Assessment of normality was made by plotting frequency distributions and confirmed using the Shapiro-Wilk test,

Analysis of covariance (ANCOVA) was used to explore change from baseline in continuous variables (e.g. visual acuity, intraocular pressure, and quality of life parameters) by treatment group [239]. In all circumstances where this was used, prior to testing, it was confirmed that the required assumptions were met to fit the model (i.e. frequency distributions were approximately normal and homogeneity of regression of baseline variables were satisfied)

Mann-Whitney U test or unpaired t-test/ANOVA was used to compare continuous outcome variables where baseline data were limited (e.g. foveal thickness and macular volume).

Binary categorical outcome variables (e.g. presence of CMO, proportion of eyes with complete retinal reattachment) were compared using the Chi-Squared test with Yates correction where >80% of observed frequencies were >5. Fishers Exact test was used where this condition was not met.

As statistical comparisons of secondary outcomes are the result of post hoc analyses, it must be acknowledged that any observed estimates of treatment effect may be imprecise.

Any deviations from these statistical methods will be further described and justified in the relevant section, as appropriate.

3.2.5 Results

3.2.5.1 *Recruitment*

Patient recruitment opened on 2nd February 2012 with the first patient recruited on 6th February 2012. The study closed at the final visit of the final patient in February 2015, 2 weeks outside the original projected timeframe.

Figure 3.4: Recruitment Line Graph

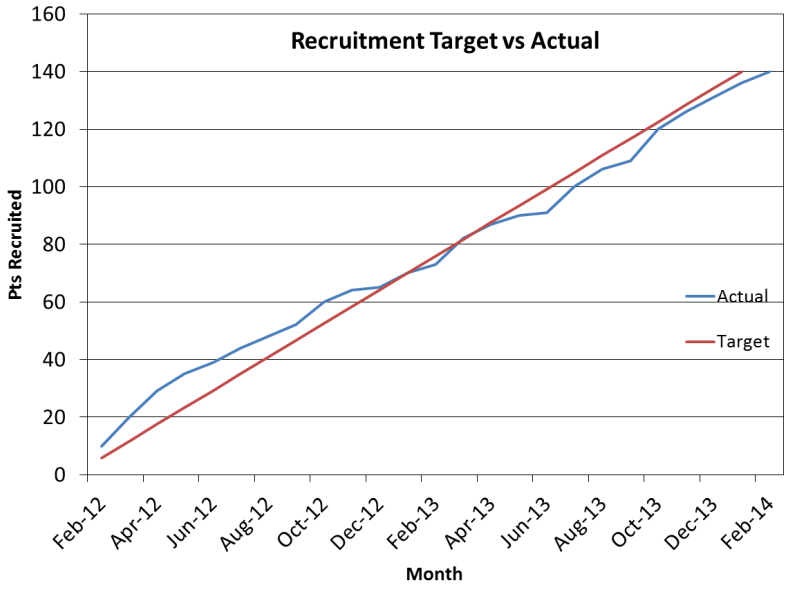


Figure 3.4 displays the recruitment line graph of projected target recruitment (red line) compared to actual cumulative rate. Rates of recruitment initially exceeded projected targets from February to December 2012. The study team met the 50% recruitment milestone of 70 patients by January 2013. Thereafter, recruitment rates fell just below target rates with recruitment completed 2 weeks after the scheduled close of recruitment in February 2014.

3.2.5.2 *Study Consort Flow Chart and Study Retention*

One hundred and ninety two patients were assessed for eligibility of which 29 were ineligible and excluded. Of the remaining 163 eligible patients, 20 patients declined to participate in the study. Three further patients enrolled in the study but were not randomized as silicone oil was not used. The remaining one hundred and forty eligible patients elected to participate in the trial and were recruited within 24.5 months of the study commencing.

Two patients in the adjunct group did not receive their first (and also second) Ozurdex implant. One patient was rendered aphakic at the time of the primary study vitrectomy. One patient was randomized prior to the surgeon's decision to use a gas tamponade and thereby became ineligible for study inclusion.

In addition to these two patients, two patients did not receive their second implant due to preoperative IOP restrictions as per the study protocol.

One patient in the adjunct group did not attend the primary assessment outcome visit and one patient in the control group was lost to follow up after month 3.

Three further patients (one adjunct and two controls) failed to complete the study at the 12 month secondary outcome assessment point. Two of these (either group) were lost to follow up. The remaining control patient was withdrawn by the study team after the 6 month outcome assessment as his treating clinician requested an injection of intraocular steroid injection thereby potentially confounding or mitigating against any potential treatment effect of the IMP.

Figure 3.5: Consort Flow Diagram for Ozurdex in PVR Study

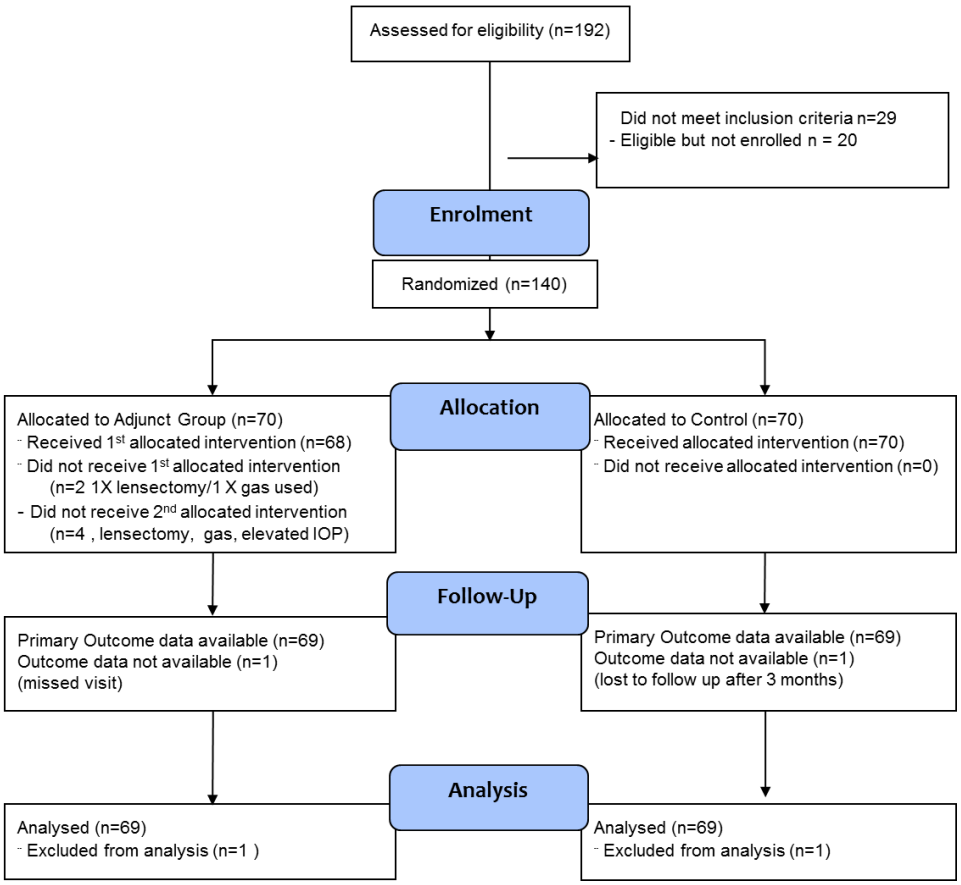


Figure 3.5 outlines the consort flow diagram. It is evident in this study that there is a high enrolment rate (89%) once screened as eligible. This is comparable to previously published studies where the median reported rate of enrolment was 90% [198]. One patient was withdrawn from the study by the trial team as he required a treatment of intravitreal corticosteroid to treat refractory cystoid macula oedema. This may have confounded any treatment effect of the IMP or negated his suitability to remain in the control group.

3.2.5.3 *Baseline Characteristics*

Baseline demographic and non-ocular characteristics are summarized in table 1 showing comparable gender, mean age, and ethnicity, with a Caucasian sexagenarian male preponderance in both groups.

Table 3.4: Non ocular Baseline Characteristics of the two treatment groups

	Adjunct Group (N=70)	**Control Group (N=70)**
Number of Patients (Eyes), n	70 (70)	70 (70)
Male/Female, n (%)	46 (65.7) / 24 (34.3)	40 (57) / 30 (43)
Mean Age in yrs,(SD)	60.6 (14.3)	61.6 (13.9)
Ethnicity:		
White, n (%)	53 (75.7)	57 (81.4)
Black, n (%)	6 (8.6)	4 (5.7)
Asian, n (%)	10 (14.3)	6 (8.6)
Other, n (%)	1 (1.4)	3 (4.3)
Scores for:		
VFQ 25, median (IQR)	66 (50, 77)	65 (55, 76)
Missing, n (%)	*1 (1.4)*	*3 (4.3)*
SF 36, median (IQR)	63 (45, 75)	72 (52, 84)
Missing, n (%)	*1 (1.4)*	*3 (4.3)*

VFQ 25 = visual functioning 25 point questionnaire, SF 36 = social functioning 36 point questionnaire, IQR interquartile range

The median refractive status in both groups was emmetropia. Approximately one third of eyes in each group (n=22 vs n=20, adjunct vs control, respectively) had not undergone previous vitreoretinal surgery, with the majority of the remaining two thirds of patients having suffered failed vitrectomy surgery with gas tamponade. Four patients in both groups had previously undergone failed scleral buckling procedures. Ten patients in the adjunct group were noted to have ocular co-morbidity limiting visual outcome compared to three control patients.

Table 3.5: Past Ocular History of the two study groups

	Adjunct Group (N=70)	**Control Group (N=70)**
Laterality (Left eye)	36 (51.4)	38 (54.3)
Refraction (SE) median (IQR)	-.6 (-5, 0)	0 (-2.63, 0)
Missing	*9 (12.9)*	*13 (18.6)*
Previous VR Surgery:		
None, n(%)	22 (31.4)	20 (28.6)
V/Gas, n(%)	36 (51.4)	36 (51.4)
V/Oil, (n%)	11 (15.7)	11 (15.7)
V/B, n(%)	0	1 (1.4)
C/B, n(%)	4 (5.7)	4 (5.7)
Median mac off episodes (IQR)	2 (1, 2)	2 (1, 2)
Co-existing ocular pathology:		
Macular Pathology n (%)	3 (4.3)	2 (2.9)
Amblyopia, n(%)	5 (7.1)	0
Corneal Scar, n(%)	0	1
Other, n(%)	2 (2.9)	0

SE = spherical equivalent, V/Gas = vitrectomy/gas, V/oil = vitrectomy/oil, V/B = vitrectomy/buckle, C/B = cryotherapy/buckle, OHT = Ocular hypertension, mac off episodes = know episodes fovea-involving retinal detachments

A summary of baseline ocular characteristics are displayed in Table 3.6. The median presenting visual acuity was zero ETDRS letters (i.e. ≤ Counting Fingers) in both groups, and mean intra-ocular pressure readings were 11.9mmHg and 13.3mmHg in the adjunct and control group, respectively. Baseline markers of inflammation and blood ocular barrier breakdown (anterior chamber cells, vitreous haemorrhage and RPE cells) were comparable between the two groups.

Thirty seven (52.9%) of the adjunct group patients were pseudophakic compared to thirty four (48.6%) control patients. Of the remainder, the majority showed signs of lens opacity with approximately ten percent of patients in each group with no cataract.

Table 3.6: Baseline Ocular Characteristics (Non Retinal)

	Adjunct Group (N=70)	**Control Group (N=70)**
ETDRS VA, median (IQR)	0 (0, 22)	0 (0, 31)
IOP, mean (SD)	11.9 (4.9)	13.3 (5.1)
***AC inflammation (cell count):**		
None (0), n (%)	38 (54.3)	33 (47.1)
Mild (1+), n (%)	30 (42.9)	29 (41.4)
Moderate (2+), n (%)	1 (1.4)	8 (11.4)
Severe (3+, 4+), n (%)	1 (1.4)	0
Lens Status:		
Clear, n (%)	8 (11.4)	7 (10)
PCIOL, n (%)	37 (52.9)	34 (48.6)
Cataract, n (%)	25 (35.7)	29 (41.4)
Vitreous Haemorrhage:		
Absent, n (%)	66 (94.3)	67 (95.7)
Present n (%)	4 (5.7)	3 (4.3)

BCVA = Best Corrected Visual Acuity, IOP= Intraocular Pressure, AC = anterior chamber, PCIOL = posterior chamber intraocular lens, ACIOL = anterior chamber intraocular lens.* AC inflammation cell count according to SUN classification [240]

A summary of baseline retinal status is displayed in Table 3.7. The fovea was detached in 60 of 70 eyes (85.7%) in the adjunct group and in 57 eyes (81.4%) in the control group. The median duration of retinal detachment was 28 and 25 days in the adjunct and control group, respectively. The median extent of retinal detachment was comparable, with eight clock hours of RD recorded in the adjunct group and nine in the control arm. Duration of RD, number of breaks and cumulative size of breaks at baseline and/or primary surgery (in eyes with previous RD repair) were similar across both groups. The median grades of anterior and posterior PVR (as assessed intraoperatively) were comparable between the two groups. Baseline foveal thickness and macular volume was unavailable for the majority of patients (117 of 140, 83.6%) and was therefore not included in the final analysis.

Table 3.7: Baseline Retinal Characteristics

	Treatment Group (N=70)	**Control Group (N=70)**
Summed Duration of RD, median (IQR)	28 (7, 45)	25 (11, 52)
Not Possible, n (%)	*17 (24)*	*21 (30)*
Clock hours of RD (Primary/Baseline), median (IQR)	6 (5, 10) / 8 (6, 11)	6.5 (5, 11) / 9 (6, 12)
Not Possible, n (%)	*7 (10) / 24 (34)*	*8 (11) / 24 (34)*
Macular status:		
Attached, n (%)	10 (14.3)	13 (18.6)
Detached, n (%)	60 (85.7)	56 (80)
Bisected, n (%)	0	1 (1.4)
PVR Grade*:		
CP, median (IQR)	3 (2, 4)	4 (2, 6)
CA, median (IQR)	4 (3, 6)	4 (4, 6)

RD = Retinal Detachment, PVR = Proliferative vitreoretinopathy, CP = posterior Grade C, CA = anterior Grade C *Measured at operation

3.2.5.4 *Operative Techniques*

Table 3.8 outlines the operative techniques employed during the primary study vitrectomy. These appear well balanced as 38 (54.3%) adjunct patients and 39 (55.7%) control patients underwent a retinectomy at the time of their primary study vitrectomy.

Table 3.8: Operative Techniques during Study Vitrectomy

	Adjunct Group (n=70)	**Control Group (N=70)**
Lensectomy	1	1
PVD Induction	5	4
PFCL n(%)	40 (57)	44(63)
Retinectomy n(%)	38(54)	39(56)
PVR Membrane Peel n(%)	42 (60)	38 (54)
Segmental Buckle, n	1	2
Retinopexy		
Endolaser n(%)	56(80)	58(83)
Cryotherapy n(%)	43(61)	48(69)

PVD = Posterior Vitreous Detachment, PFCL = Perfluorocarbon

3.2.5.5 *Primary Outcome Result*

Primary outcome data were available for 69 out of 70 patients in each group. One patient in the control group was lost to follow up after month 3 and one patient in the adjunct group was prematurely withdrawn as they had failed primary surgery and no month 6 data were collected. It was subsequently agreed by both the TSC and DMC that they remain in the study and month 12 data were collected.

Table 3.9: Primary Outcome Result (Available ITT analysis)

	Adjunct Group (N=69)	**Control Group (N=69)**	**Effect Estimate Odds Ratio(95% CI)**
Proportion of patients satisfying primary outcome measure, % (95% CI)	49 (37, 62)	46 (34, 59)	0.89 (0.46, 1.74)

There was no observed difference in primary outcome between the two groups (Table 4): 49.3% of patients (n= 34 of 69) in the adjunct group achieved a stable retinal reattachment with silicone oil removal without additional vitreoretinal surgical intervention at 6 months, compared to 46.3% (n=32 of 69) in the control group. (Odds Ratio 0.89, 95% Confidence interval 0.46 – 1.74, p= 0.733 Chi Squared). Best case and worse case imputation analysis did not affect the primary outcome findings. Sub-group analysis stratifying by severity of PVR (Grade CP or CA > 4) did not show any statistically significant difference in primary outcome achievement.

3.2.5.6 *Visual Outcomes*

3.2.5.6.1 Vision by predefined secondary outcome

At six months following study vitrectomy (Table 3.10) mean visual acuity was 38.3 ETDRS letters (standard deviation 23.7) in the adjunct group compared to 40.2 letters (standard deviation 21.1) in the control group (p=0.898, ANCOVA) . This mean visual acuity approximates to a LogMAR visual acuity of 0.9 and a Snellen Equivalent of 6/48. Similarly, change from baseline showed no significant difference in the two treatment groups. A sensitivity analysis excluding eyes with pre-existing ocular co-morbidity limiting visual outcome (10 eyes excluded in adjunct group, 3 in control group) was performed and did not significantly affect the findings. The proportion of eyes achieving a visual acuity ≥ 55 ETDRS letters was also comparable, with 21 of 69 eyes (30.4%) in the adjunct group achieving this vision or better, compared to 17 of 69 eyes (24.6%) in the control group.

Table 3.10: Visual Outcome at 6 months

	Adjunct Group (N=69)	**Control Group (N=69)**	**Effect Estimate (95% CI)**
ETDRS BCVA, mean (SD)			
- **At 6 months**	38.3 (23.7)	40.2 (21.1)	-
- **Change from baseline at 6 months***	24.5 (28.6)	23.1 (26)	1.1 (-6.3, 8.4)
Proportion of patients achieving ETDRS VA ≥ 55, n (%)	21 (30)	17 (25)	
Sensitivity Analysis	**(N=59)**	**(N=66)**	
ETDRS BCVA, mean (SD)			
- **At 6 months**	41.60 (23.1)	41 (20.9)	-
- **Change from baseline at 6 months***	26.4 (29.3)	23.2 (26.4)	-1.2 (-8.8, 6.4)

* Adjusted for respective baseline, BCVA = Best Corrected Visual Acuity,

3.2.5.7 *Exploratory analysis of visual outcome in eyes without limited visual potential*

Further exploratory analysis of the subgroup included in the aforementioned sensitivity analysis (i.e. 125 eyes with no known pre-existing ocular morbidity limiting vision) was performed comparing: a) visual acuity at each study visit and b) VA change from baseline (proportion gaining/losing letters)

3.2.5.7.1 Visual outcomes by time-point

The mean visual acuity adjusted for baseline was calculated and statistical comparisons made (ANCOVA). Confirmation that the required assumptions for the model were met (i.e. approximately parametric distributions and homogeneity of regression was present across baseline variables by treatment group).

The adjusted mean VA in the adjunct group was higher than the control group at all timepoints from Day 10 until month 6 when the values become comparable. The adjusted mean VA in the adjunct group at Day 60 post primary vitrectomy was 44.6 letters (95% confidence interval 40.2 to 49.1) compared to 37.9 letters (95% CI 33.7 to 42.2) in the control group. This was statistically significant. (p = 0.034 ANCOVA). Table 3.11 displays the adjusted mean vision in each group (with 95% CI) at each scheduled study visit up to month 6.

Table 3.11: Adjusted Mean Visual Acuity over Study Period

Study Visit	Adjunct (n=59)	Control (n=66)	p value
Baseline	15 (9.3 to 21.1)	17 (11.9 to 23.0)	0.575
Day 10	39 (34.3 to 43.6)	34 (29.8 to 38.6)	0.149
Week 4	43 (37.9 to 47.7)	36 (31.7 to 41.1)	0.063
Day 60	**45** (40.2 to 49.1)	**38** (33.7 to 42.2)	**0.034**
Month 3	44 (39.4 to 49.0)	38 (33.9 to 42.8)	0.079
Month 6	41 (36.4 to 47.4)	42 (35.5 to 45.9)	0.099

Adjusted mean VA to nearest integer (95% Confidence Interval) ANCOVA p value, with prior confirmation that assumptions for model are met

Figure 3.6: Mean Visual Acuity by Group over Study Period

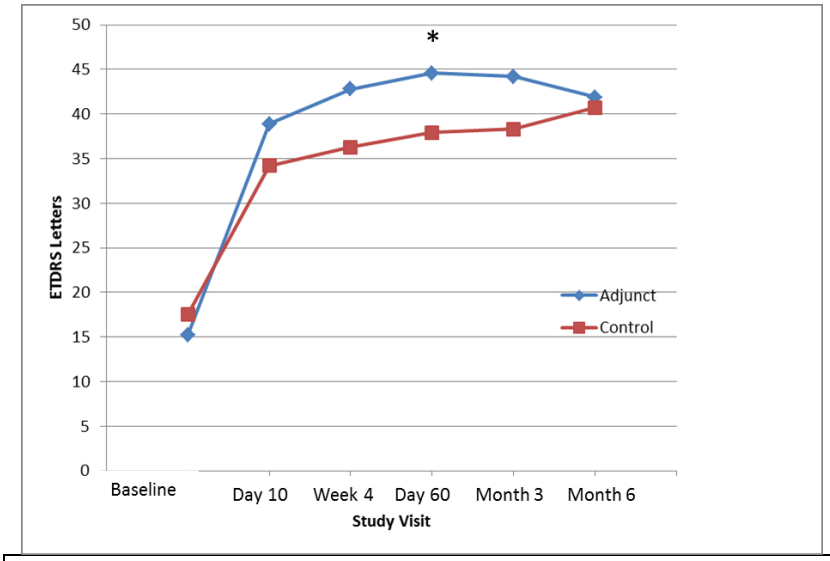


Figure 2.1 displays the adjusted mean VA by treatment group over the 6 month study period. VA was higher in the adjunct group at all timepoints from day 10 but becomes comparable at month 6; * denotes statistical significance at Day 60 (95% CI displayed in Table 3.11 above)

Figure 3.7: Box plots of Visual Acuity at Day 60 and Month 6

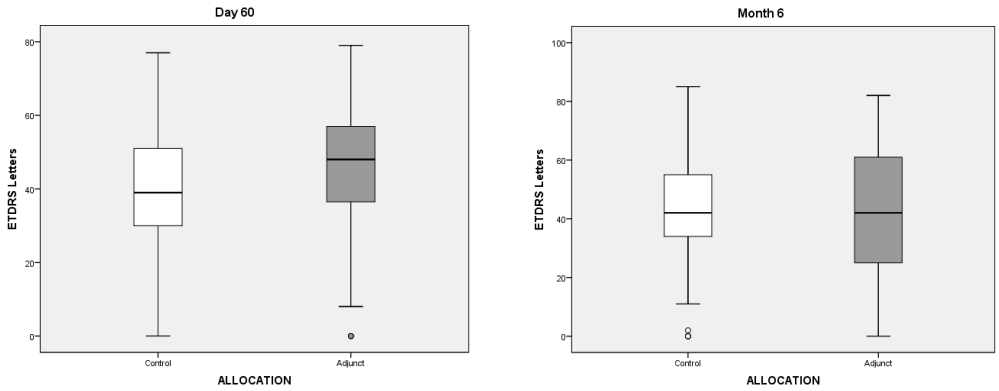


Figure 3.7 displays the visual acuity distribution of both groups at Day 60 and month 6 in sub group analysis of 125 eyes (59 adjunct, 65 control). The adjusted mean VA was higher in the adjunct group at Day 60 where the mean VA is 45 letters compared to 38 letters in the control group. The vision returns to comparable levels at the primary outcome endpoint at month 6. (central bar = median, box = interquartile range, whiskers = range)

3.2.5.7.2 Visual Outcome by proportion of letters gained or lost

Visual outcome was categorised into proportion of eyes gaining or losing letters when measured at six months in comparison to baseline. As previously reported, median baseline vision was Zero ETDRS letters in both groups.

Table 3.12: The proportion of eyes gaining letters from baseline

ETDRS LETTERS GAINED	ADJUNCT (n= 59)	CONTROL (n=66)
GAINED ≥ 10 letters	42 (70.0)	48 (72.7)
GAINED ≥20 letters	38 (63.3)	42 (63.6)
GAINED ≥ 30 letters	31 (51.7)	36 (54.5)
GAINED ≥ 40 letters	21 (35.0)	24 (36.4)
GAINED ≥ 50 letters	16 (26.6)	10 (15.2)
GAINED ≥ 60 letters	9 (15.0)	3 (4.5)

Data expressed as n of 59 (%) in adjunct group and n of 66 (%) control group

The proportion of eyes gaining 10, 20, 30 and 40 ETDRS letters from baseline visual acuity is comparable between the two groups (Table 3.12). However, the proportion of patients gaining 60 or more letters is 15.0% (n=9) in the adjunct group compared to only 4.5% (n=3) in the control group.

Eleven patients (18.3%) in the adjunct group lost vision compared to nine (13.6%) in the control group. The proportion of patients suffering mild visual loss (≤ 10 letters) was 3.3% (n=2) in the adjunct group compared to 6.1% (n=4) in the control group. Severe visual loss (≥ 20 letters) was more common in the adjunct group (10%, n=6) compared to controls (4.5%, n=3).

3.2.5.8 *Secondary Anatomical Outcomes*

At 6 months, the proportion of patients achieving complete retinal reattachment or a stable posterior retinal reattachment was comparable between the two treatment groups (Table 3.13). Similarly, the proportion of patients with a tractional retinal detachment at 6 months was also comparable. The rate of overt PVR recurrence (defined as the presence of postoperative PVR at any time-point up to 6 months post study vitrectomy) was 58.0% (n= 40) in the adjunct group and 59.4% (n=41) in the control group.

There was no observed difference in the number of operations to achieve primary success (as defined in the primary outcome measure); however, 11 patients (15.9%) underwent more than one operation to achieve success in the control group compared to 3 patients (4.4%) in the adjunct group.

Table 3.13: Secondary anatomical outcomes

	Adjunct Group (N=69)	**Control Group (N=69)**	**Effect Estimate Odds Ratio (95% CI)**
Overt PVR recurrence*, n (%)	40 (57)	41 (59)	
Complete retinal reattachment **, n (%)	37 (53.6)	43 (62.3)	0.699,(0.36 – 1.38, p= 0.301)
Stable posterior retinal reattachment ** n (%)	46 (66.7)	48 (69.6)	
TRD **, n (%)	15 (22)	13 (19)	
Number of procedures to achieve attachment, n (%) **0** **1** **2**	 41 (59.4) 25 (36.2) 3 (4.4)	 37 (53.6) 21 (30.4) 11 (16)	

* Between the primary study vitrectomy and 6 months, ** without silicone oil in situ

3.2.5.9 *Macular Findings*

Each parameter will be reported sequentially to elaborate further but in brief the proportion of eyes with macular oedema in the adjunct group was 42.6% (n=29) compared to 65.2% (n=45), (p= 0.007, Chi Squared). Similarly, the proportion of eyes with a foveal thickness >300µm in the A1 macular subfield was lower in the adjunct group (n=30, 47.6 %) compared to the control group (n=42, 67.6%) (p= 0.036), Chi Squared). The median foveal thickness was lower in the adjunct group (297µm and 8.85mm^3) compared to the control group (365µm and 9.23 mm^3).

Table 3.14: Summary of Macular Findings at Month 6

	Adjunct Group (N=69)	**Control Group (N=69)**	**Effect Estimate Odds Ratio (95% CI, p value)**
*** CMO present**	29 (42.6)	45 (67.2)	0.36 (0.17 to 0.78, p= 0.007)
**** FT > 300 µm, n (%)** ***Missing, n (%)***	30 (47.6) *6 (9)*	42 (67.7) *7 (10)*	0.43 (0.20 to 0.95, p=0.036)
FT, median (IQR) - **At 6 months** ***Missing, n (%)***	297 (255, 380) *6 (9)*	365 (284, 455) *7 (10)*	(P=0.053)
Macula pucker/ERM †, n (%)	40 (57)	41 (58.6)	
ERM surgery†, n (%)	33 (47)	31 (44.3)	

* % expressed as proportion of available cases (68 eyes in adjunct group 67 eyes control group), ** % expressed as proportion of available cases (63 eyes adjunct group, 62 eyes control) † % expressed as proportion of n=70

3.2.5.9.1 Cystoid macular oedema

The presence of cystoid macula oedema was defined as either intraretinal cystic spaces visible on spectralis OCT and/or the characteristic clinical appearance on fundal assessment by slit-lamp biomicroscopy. Outcome data for this variable was available in 67 eyes in the control group and 68 eyes in the adjunct group. An assessment of the presence of CMO either clinically or by SD-OCT was not possible in two patients in the control group and one in the adjunct group due to media opacity. One patient was lost to follow-up in the standard group and one adjunct patient was prematurely withdrawn from the study prior to this data collection point.

At 6 months post study vitrectomy, cystoid macula oedema was present in 67.1% (n=45) of patients in the standard group compared to 42.6% (n= 29) in the adjunct group (Odds Ratio 0.36, 95%CI 0.17 to 0.78, p= 0.007 Chi Squared with Yates correction). This observed difference was statistically significant (Table 3.14).

3.2.5.9.1.1 Exploratory Cystoid Macular Oedema and Visual Acuity

At month 6 there was no difference in mean visual acuity by treatment group (38.3 vs 40.2 ETDRS letters adjunct vs control).However, a statistically significant difference was found between rates of CMO by treatment group.

Therefore, a comparison was made between the visual outcomes achieved by treatment group depending on the presence or absence of CMO at 6 months. This was performed on the 123 of 125 eyes after excluding the 13 eyes with limited visual recovery and 2 eyes where CMO data was missing (refer section3.2.5.6.1)

In the standard group, at 6 months, the adjusted mean VA in eyes with CMO (n=44) was 38.5 letters compared 50.4 letters in those without (n=20) (95% CI 33.2 to 43.8 vs 42.4 to 58.4, no CMO vs CMO, respectively, p=0.017 ANCOVA).

In the adjunct group, at 6 months, the adjusted mean VA in eyes with CMO (n=25) was 30.1 letters compared to 51.2 letters in those without (n=34). (95% CI 22.0 to 38.2 vs 37.1 to 51.9 no CMO vs CMO, respectively, p=<0.001 ANCOVA).

3.2.5.9.2 Foveal thickness

Spectral Domain – OCT derived values for central foveal thickness (FT) were recorded for patients at all visits where possible, and a comparison at the 6 month time-point was made. As baseline parameters were unavailable in the majority of patients a comparison between the changes in foveal thickness from baseline was not indicated. Therefore, cross sectional comparisons were made between the two groups at 6 months.

OCT-derived data was available for 62 of 70 patients in the control group and 63 of 70 patients in the adjunct group. Reason for missing data was most commonly poor quality image acquisition due to either media opacity or poor central fixation.

In the control group, the median FT was 365.0 um with an interquartile range of 168.5um. The median FT in the adjunct group was 296.5um with an interquartile range of 107.75um. Mann Whitney U test for unpaired non-parametric data marginally failed to achieve statistical significance. (p=0.054)

A comparison between the proportion of eyes in whom the central A1 macular subfield was >300um was investigated as a predetermined secondary outcome. In the control group, 42 of 62 eyes (67.7%) were found to have a central foveal thickness of >300um, compared to 30 of 63 (47.6%) of those in the adjunct group. Binomial logistic regression determined an odds ratio of 0.43 (95 % CI 0.20 to 0.95, p=0.036). This achieved statistical significance using the Chi squared test with Yates correction (p= 0.036).

3.2.5.9.3 Macular pucker formation and surgery

Forty patients (57.1%) in the adjunct group and 41 patients (58.6%) in the control group developed macular ERM at any time-point up to 6 months, with comparable rates of macular pucker surgery between the two groups.

3.2.5.10 *Cataract*

The proportion of phakic patients in the adjunct group who underwent cataract surgery during the six months after the study intervention was 75.8% (n=25 of 33), in the adjunct group compared to the 86.1% in the control group (n=31 of 36). The definition of cataract as an adverse event has been described and the significance of this will be discussed further in section 3.2.9.

3.2.5.11 *Quality of Life Parameters*

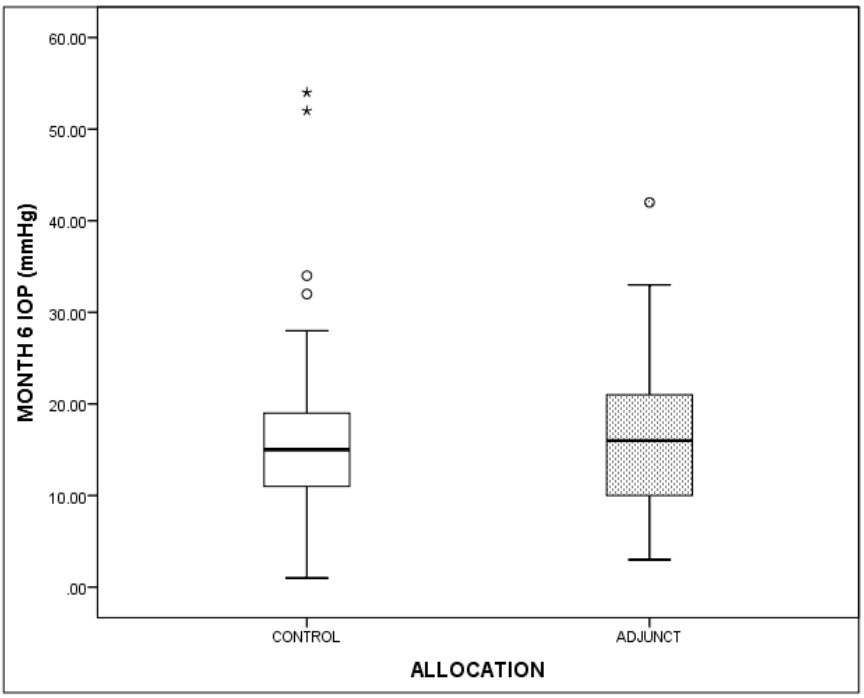
The Social Functioning 36 (SF-36) questionnaire and National Eye Institute Visual Functioning Questionnaire (NEI-VFQ 25) were validated tools used to assess quality of life (QAL) of participants in the trial. The aforementioned questionnaires were administered to all participants at baseline and again at the primary endpoint, 6 months post study vitrectomy. Additionally, a simple three question screening tool for depression was used with flags for active symptoms of severe depression included, to prompt further action as required.

There was no statistical difference between the mean VFQ 25 score or SF6 score at 6 months between the two groups, when controlling for baseline scores (p=0.995 and p=0.158, respectively, Mann Whitney U).

3.2.5.12 *Intraocular Pressure Outcomes – Elevated IOP*

A higher proportion of patients in the adjunct group (45.7%, n=32) experienced at least one episode of elevated IOP (>25mmHg) at any time-point up to 6 month post study vitrectomy compared to the control group (31.4%, n=22), although this was not statistically significant (p=0.135, Chi Squared). Furthermore, there was no statistical difference in the comparison of estimated means between the two groups at the 6 month time-point. (p= 0.282, ANCOVA).

Figure 3.8: Box plot of Intraocular Pressure at Month 6



At 6 months the mean IOP was 15.8mmHg (SD of 9.53) in the control group compared to 16.7mmHg (SD of 8.40) in the adjunct group (Figure 3.8). There was no statistical difference in the comparison of estimated means between the two groups at this 6 month time-point. (p= 0.282, ANCOVA test) (centre line = median, box = interquartile range, whiskers = range)

Figure 3.9 displays the IOP fluctuation over time throughout the first 6 month study period. The mean IOP at baseline was 13.3mmHg (standard deviation 5.13) in the control group and 11.9mmHg (standard deviation 4.92) in the adjunct group. Mean IOP peaked at Day 10 post study vitrectomy in both groups (20.3mmHg vs 21.9mmHg; control vs adjunct, respectively) and was at its lowest at the Day 1 post removal of oil time-point (12.7mmHg vs 11.0mmHg; control vs adjunct, respectively). At 6 months the mean IOP was 15.8mmHg (SD of 9.53) in the control group compared to 16.7mmHg (SD of 8.40) in the adjunct group.

Figure 3.9: Line graph of Mean IOP by Treatment group

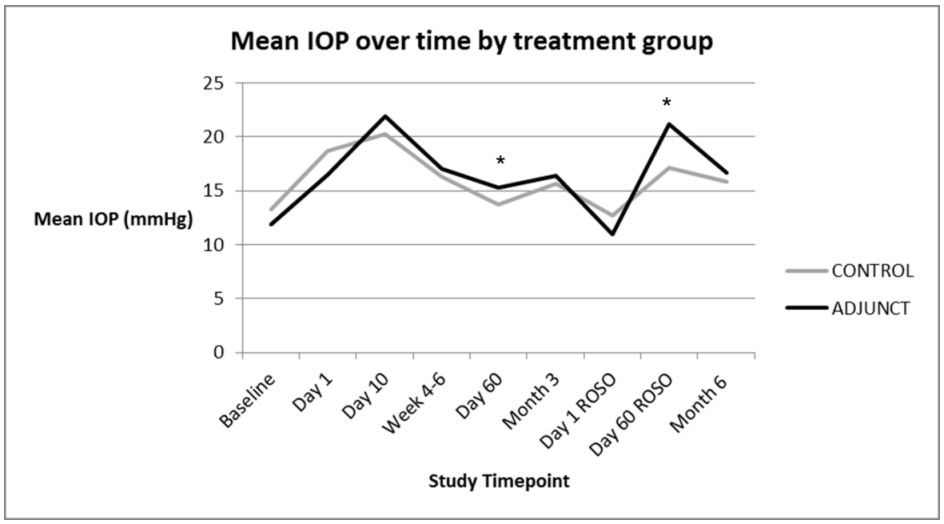


Figure 3.9. Line graph of mean IOP fluctuation. No statistical differences were found on comparison of means between the groups for the following timepoints; day 1(p=0.056), day 10 (p=0.22), week 4(0.377), month 3 (p=0.38), day 1 post ROSO (p= 0.31) or Month 6. Mean IOP at day 60 post study vitrectomy and day 60 post removal of oil were significantly higher in the adjunct group compared to the control group (* indicates statistically significant difference)

Figure 3.10: Boxplots of Adjusted Mean IOP Day 60 after study vitrectomy and oil removal

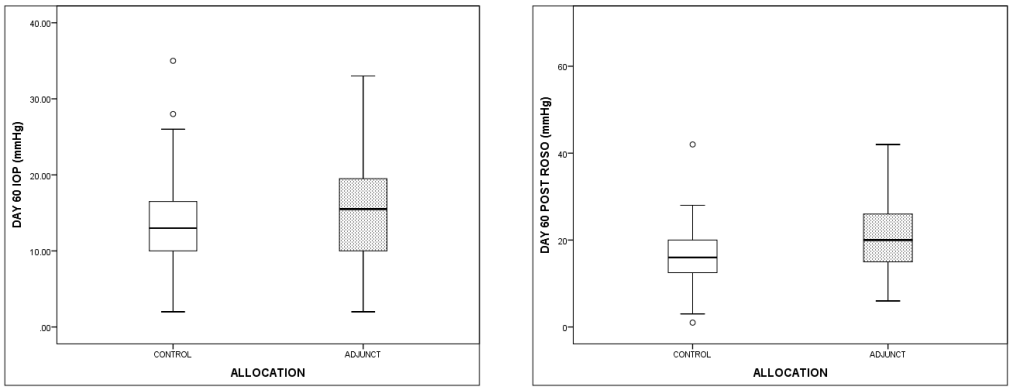


Figure 3.10 displays the box plots of distribution of IOP 60 days after the primary study vitrectomy time-point (when 1st Ozurdex implant was injected in adjunct group) and Day 60 following removal of silicone oil (ROSO) Estimated means at Day 60 are 13.3mmHg , 95%CI 12.0-14.9 vs 15.6mmHg, 95% CI 14.2-17.1 (p=0.036) , day 60 post ROSO estimated means are 16.8 (95% CI 14.1 - 19.4) in controls vs a21.6 (95% CI 18.8-24.3)in adjunct group (p= 0.015). (central bar = median, box = interquartile range, whiskers = range)

No patients were using ocular hypotensive agents at the time of their primary study vitrectomy. In total, there were 81 episodes of elevated IOP in the adjunct group compared to 70 episodes in the control group. Of these, 42 vs 60 events were mild, 23 vs 17 events were moderate and 5 vs 4 events were severe, in the control vs adjunct group, respectively.

An equal number of patients (42 of 70, 60.0%) per group required treatment with at least one ocular hypotensive agent at any time-point during the 6 month period. More patients in the control group (18 of 69 (26.1%)) remained on ocular hypotensive therapy at month 6, compared to 8 of 69 (11.6%) in the adjunct group. Fewer patients in the adjunct group (5 of 70) had an episode of elevated IOP which required treatment with a systemic ocular hypotensive agent, than in the control group (8 of 70). 5 patients in the control group required laser or surgical intervention to control the IOP compared to 4 in the adjunct group.

3.2.5.13 *Intraocular Pressure Outcomes - Hypotony*

Rates of hypotony (defined as an IOP <6mmHg) were comparable across the two groups with 14 patients (20.0%) suffering at least one episode in the adjunct group compared to 17 patients (24.3%) in the control group. Just under one half (48%) of all episodes of hypotony were recorded at Day 1 post removal of oil.

3.2.6 Adverse Events

There were a total of 595 reported adverse events (AE) and 17 serious adverse events (SAE). No serious adverse reactions (SAR) or suspected unexpected adverse reactions (SUSAR) were reported over the study period. The proportion of patients suffering at least one AE was similar between the two groups; 66 of 70 (94.3%) adjunct patients and 63 of 70 (90%) control patients.

3.2.6.1 *Non Serious Adverse Events*

	Adjunct Group (N=70)	Control Group (N=70)
Total number of AEs, n (%)	285 (96.6)	310 (97.8)
Number of expected AEs:		
Cataract, n (%)	0 (0)	1 (0.42)
Raised IOP, n (%)	85(39.2)	75 (31.4)
Hypotony, n (%)	27 (12.4)	31 (13)
Sterile Hypopyon, n (%)	0 (0)	1 (0.4)
Retinal Detachment, n (%)	45 (20.7)	51 (21.3)
Uveitis, n (%)	10 (4.6)	24 (10)
Further Surgery, n (%)	41 (18.9)	51 (21.3)
Glaucoma, n (%)	3 (1.4)	2 (0.8)
Headache, n (%)	5 (2.3)	1 (0.4)
Migraine, n (%)	1 (0.5)	2 (0.8)
Vitreous Opacities, n (%)	0 (0)	0 (0)
Tractional Maculopathy, n (%)	0 (0)	0 (0)
Number of unexpected AEs		
Systemic Illness, n (%)	15 (22)	18 (25.4)
Ocular Vascular Occlusion, n (%)	3 (4.4)	3 (4)
Raised Blood Pressure, n (%)	6 (8.8)	6 (8.5)
Bombe, n (%)	6 (8.8)	6 (8.5)
Fellow Eye RD Surgery*, n (%) ***Number of patients, n (%)***	2 (2.9) *1 (1.5)*	1 (1.4) *1 (1.6)*
Other (Ocular), n (%)	10 (14.7)	17 (23.9)
Other (Non-Ocular), n (%)	26 (38.2) / 0 (0)	20 (28.2)

(Percentage calculated in relation to total number of AE/SAE in each group – Overall 595)

3.2.6.2 *Serious Adverse Events*

SAEs were similarly distributed between the two groups, with none deemed to be related to the study IMP.

	Adjunct Group (N=70)	Control Group (N=70)
Total number of patients with at least one SAE, n (%)	7 (10)	6 (8.6)
Total number SAEs, n (%) *(Percentage calculated in relation to total number of AE/SAE in each group – Overall 595 / 17)*	10	7
Number of unexpected SAEs		
Systemic Illness, n (%)	9 (90)	4 (57)
Other (Ocular), n (%)	1(10)	0 (0)
Other (Non-Ocular), n (%)	0 (0)	3 (42.9)

There was one ocular SAE in the adjunct group as one patient was admitted for a corneal suture –related abscess with suspected secondary endophthalmitis. The event was deemed unlikely related to the IMP and closed.

3.2.6.3 *Nested studies prompted by observed ocular adverse events*

Throughout the trial, a number of unexpected events were observed. These formed the basis of valuable learning points on a personal level, and in the wider field, which were deemed significant for publication. Two selected clinical observations will herein be described as nested studies which arose during the study period. Thereafter, the main discussion of the trial findings will follow. These nested studies have also generated findings which will be included in the overall discussion of this chapter (section 3.2.7.2.2).

3.2.6.3.1 Nested Study 1; neurotrophic corneal ulceration after PVR surgery

Five study participants suffered an AE deemed related to the surgical repair and not related to the IMP. These events had not been previously reported in the literature and hence were successfully published as a series in *JAMA Ophthalmology* [241]. They will be briefly described as a case series in sequence followed by a short discussion, given their relevance to this thesis. Four were control patients and one had received the adjunctive therapy.

3.2.6.3.2 Case 1

A 68 year-old South Asian male developed a central epithelial defect with a secondary infective crystalline keratopathy 6 weeks after his study vitrectomy. He was treated with an intensive topical regimen using a fluoroquinolone. Corneal anaesthesia was documented 4 weeks later and a differential diagnosis of herpetic keratitis considered with additional topical and systemic anti-viral therapy instituted. After a negative viral PCR and a lack of treatment response, he was switched to intensive topical lubricants. A botulinum toxin- induced ptosis aided resolution at 6 months, but with 20% central corneal thinning and marked stromal scarring.

3.2.6.3.3 Case 2

A 58 year old Caucasian male with dense strabismic amblyopia developed a neuropathic ulcer eight weeks post ROSO and presented to his local ophthalmic unit (Figure 3.11 C). He was treated with systemic antivirals, in addition to topical antibiotics and infrequent lubricants. One week later, upon review at MEH, systemic therapy was discontinued and resolution was achieved at three weeks with intensive lubricants, with residual central stromal scarring and 30% thinning.

3.2.6.3.4 Case 3

A 72 year-old Caucasian female required augmented barrier endolaser at the time of silicone oil removal. Ten weeks later she developed a neurotrophic corneal ulcer. Resolution was achieved after three weeks of prophylactic topical antibiotics and intensive topical ocular lubricants, with resultant mild stromal thinning and residual scarring in the visual axis.

3.2.6.3.5 Case 4

A 58 year-old Caucasian female developed a painless paracentral corneal epithelial defect (Figure 3.11 A) and fixed dilated pupil was noted five weeks post study vitrectomy. Complete resolution was achieved after three weeks with combined topical antibiotics and intensive topical ocular lubricants.

3.2.6.3.6 Case 5

After three failed previous procedures, a 53 year old Caucasian female presented with a painless reduction in vision ten weeks post study vitrectomy and was diagnosed with a neurotrophic ulcer. Complete resolution was achieved with four weeks of prophylactic topical antibiotics, intensive lubricants and a switch to an unpreserved topical steroid.

All five eyes were treated with standard Argon (532nm) endolaser settings (200-250 mW, 0.2s and 0.2s). Fundus examination showed marked confluent chorioretinal scarring at 3 and 9 o' clock in each case (Figure 3.11 B and D). We concluded that confluent intraoperative endolaser at these sites compromised long ciliary nerve function, with resultant corneal anaesthesia and ulceration. Concurrent short ciliary nerve damage may have occurred in case 4 which also had mydriasis. No other clinical signs suggested a lesion elsewhere in the trigeminal nerve, nor a polyneuropathy.

Figure 3.11: Composite of neurotrophic keratitis after retinal detachment surgery with PVR

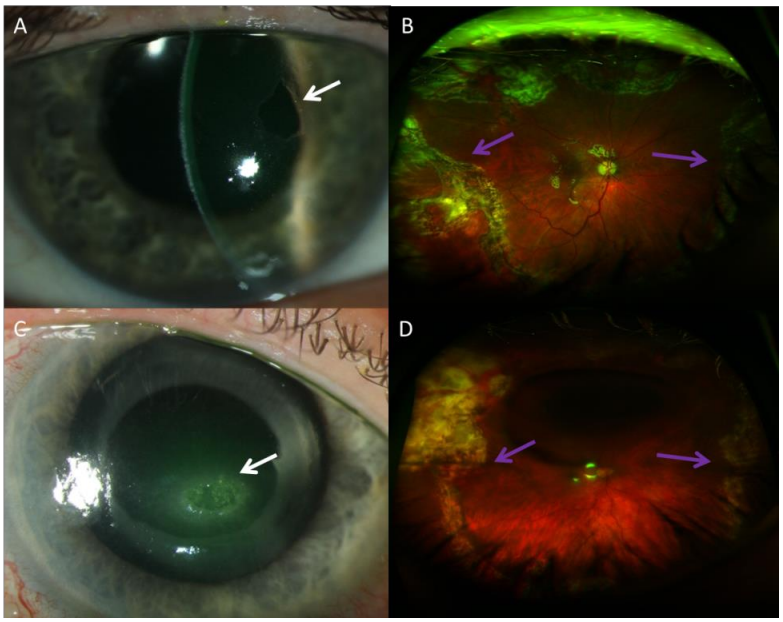


Figure 3.11. Composite images of AP slit lamp photographs (A) and (C) with corresponding fundus images of cases 4 and 2 with corresponding fundus images. White arrows indicate corneal ulceration; purple arrows indicate confluent laser scars at site of long ciliary nerves. We observed a more favourable outcome with a prompt diagnosis compared to when alternative causes were initially misdiagnosed.

3.2.6.3.7 Discussion of events; nested study 1

As neurotrophic keratopathy following transcleral cyclodiode laser is well known [242] treatment in the regions of the long ciliary nerves is avoided. Retinal surgeons are not afforded the same luxury as 'treatment-immune sites' as confluent retinopexy may be required for sustained retinal reattachment. Retinal laser-induced internal ophthalmoplegia has been reported in diabetic patients following diode photocoagulation [243] and corneal sensitivity reduction after argon retinal laser [244]; the former thought to be short ciliary nerve damage and the latter damage to the long ciliary nerves.

We noted a more favourable outcome with early diagnosis (cases 3, 4 and 5). Where alternative underlying causes were initially entertained (cases 1 and 2), the outcome appears to have been less favourable.

This first reported series highlighted the importance of remaining mindful of the long ciliary nerves intraoperatively and where possible avoiding heavy confluent treatment at these sites, without compromising adequate retinopexy. It stressed the importance of prompt recognition and treatment of corneal anaesthesia in order to minimise the risk of ulceration and visual loss.

3.2.6.4 *Nested study 2; implant position in silicone oil*

3.2.6.4.1 Background

The pharmacokinetics of Ozurdex in vitrectomised eyes in both animal and human models has been previously discussed. (section 3.1.2.1) The unpublished *in vitro* studies of its pharmacokinetics in the immiscible mixture of saline and silicone oil by the product's manufacturer have also been previously outlined.

However, the clinical experience of the slow release dexamethasone implant in silicone oil-filled eyes remains limited. In particular, implant behaviour in this atypical intraocular environment has not been described.

In 2013, a case report of a trapped implant in the macula region of an oil-filled eye reported an associated epiretinal membrane formation and advised either its prompt surgical removal or an attempt to displace the implant through posturing[245]. We had not observed any similar adverse sequelae despite frequently noting the implant positioned behind the oil bubble postoperatively. We therefore reported a different experience of this which was published in response [246].

An internal safety review of trial patients who had received the implant was conducted in order to determine the incidence of trapped implants and elaborate further on any associated adverse effects of such an occurrence at the request of the sponsor.

As the trial remained in active recruitment at the time of the 2013 publication, a retrospective review of available cases (n=55) was performed and submitted to the sponsor. However, a complete review of all sixty eight eyes of sixty eight patients in whom the IMP had been administered, was performed upon study closure and is herein included in this thesis.

As previously described, the Ozurdex® implant was injected through an open sclerotomy port prior to closure in the oil-filled eye of sixty eight patients included in the treatment arm of this study. The implant injection procedure was carried out according to study protocol in all cases. A descriptive study of implant position from the time of intraoperative injection until dissolution is herein described.

3.2.6.4.2 Method

Implant position was observed by slit lamp fundal examination at specific time-points and recorded in the medical notes contemporaneously; day one, day 10, 1 month and 3 months, post-operatively. A retrospective review of its position in relation to the retinal surface was performed and subsequently categorised into one of three zones (Figure 1): any part of the implant within the temporal arcade or abutting the optic disc (zone1); outside zone 1, but posterior to the equator (zone 2); anterior to the equator (zone 3). Spectralis domain optical coherence tomography was attempted at all visits in accordance with the study protocol.

Figure 3.12: Schematic Representation of Zones of Implant Position

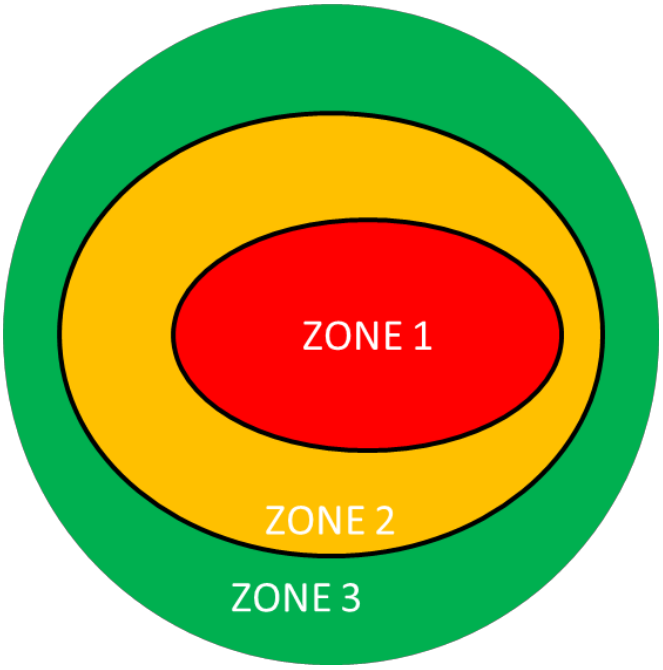


Figure 3.12 shows a schematic representation categorising the implant position in relation to the retinal surface. An implant in zone 1 was within the arcade or abutting the optic disc. Zone 2 implants were within the equator but outside zone 1. Zone 3 implants were anterior to the equator

Baseline demographics and ocular characteristics were recorded. More specifically, the following ocular characteristics were considered to be potentially clinically relevant and hence were also recorded: the preoperative presence of epiretinal membrane, history of macular ERM/ILM peel or intraoperative peel, pre-existing or intraoperative vitreous haemorrhage, intraoperative use of perfluorocarbon (PFCL). Day 1 IOP was used as a surrogate marker for oil fill.

Univariate logistic regression was used to identify factors that might be predictive of immediate and final implant position. Since none of the independent variables were significant in univariate models, we did not proceed to a multiple variable regression analysis.

3.2.6.4.3 Results:

3.2.6.4.3.1 Baseline Characteristics

Sixty eight eyes of sixty eight patients were eligible for the inclusion in this nested study. The mean age at the time of surgery was 60 years and 4 months (standard deviation 14.3). Fifty one (75.0%) patients were Caucasian, ten (14.7%) south Asian and six (8.8%) African/Caribbean. There was an equal distribution of laterality. Premorbid refractive status was known in 64 of the 68 patients. Of these, approximately one half (51.6%) of eyes were within one dioptre of emmetropia in terms of spherical equivalent. Seven eyes (10.9%) had refractive error (spherical equivalent) of ≥ 6 dioptres. Surgery for primary PVR (i.e. no previous retinal detachment surgery) was performed in 20 patients (29.4%), with 27 (39.7%) patients having previously undergone one operation and the remaining 21 patients two or more previous operations (median number of previous operations is 1 range 0-3). There were an equal number of phakic and pseudophakic patients at the time of surgery when the implant was injected. Forty patients (58.8%) underwent an intraoperative macular peel (either ERM alone or combined ILM/ERM peel) with an additional six patients having previously undergone macular peeling surgery. Perfluorocarbon was used as in intraoperative surgical tool in 40 of the 68 eyes (58.8%). Vitreous haemorrhage was present in four eyes and the median grade of posterior PVR was CP 2 (range 0-12, IQR CP 0-3).

3.2.6.4.3.2 Implant Behaviour Intraoperatively

No formal assessment of implant trajectory or velocity was conducted for the purposes of this study. Some groups have investigated the velocity of the implant in varying media, comparing its speed in saline and in vitreous [247-249] . Reported rates vary from 0.8m/s to 1.2 m/s in saline, with velocity reduced in vitreous due to increased impedance. There have not been any reports in the literature to date investigating these parameters in the oil-filled eye.

We encouraged the operating surgeon to visualise the tip of the injector such that it could be angled towards the mid-vitreous cavity, thereby allowing the longest intraocular path and theoretically reduce the risk of resultant local tissue trauma. The procedure for injecting the implant has been described in Section 3.2.4.3.1 and visualised in Figure 3.3.

In reality, the distance that the implant travelled within the silicone oil bubble was relatively short. The viscosity of silicone oil is greater than saline and vitreous (which is 99% water) and hence impedance greater. We anecdotally found that the implant consistently halted a very short distance from the tip of the applicator. Thereafter, due to its increased density and lipid insolubility, it ‘sank’ to the most dependant part of the eye (usually the posterior pole in the supine patient). No further attempts to visualise the implant were made until the next day slit lamp postoperative assessment at day 1.

3.2.6.4.3.3 Postoperative Findings

3.2.6.4.3.3.1 Exceptions to the Zone Category

Two implants were situated in positions outside Zones 1 to 3 at Day 1 as follows:

Case 1:

A pseudophakic, 53 year-old Caucasian female underwent the study vitrectomy following a failed previous primary repair. At Day 1, the steroid implant was trapped behind the posterior chamber intraocular lens/bag complex and anterior to the silicone oil bubble. The implant position was unchanged at a routine ten day postoperative visit (Figure 3.13). The implant was confirmed to have spontaneously dislocated to the vitreous base at one month post injection (zone 3) after the patient had reported its disappearance from the pupillary axis ten days earlier. No adverse effect was noted. The patient had a routine removal of oil procedure four months postoperatively and was subsequently discharged from the vitreoretinal service with an attached retina at twelve months. [250]

Figure 3.13: Retro-IOL implant

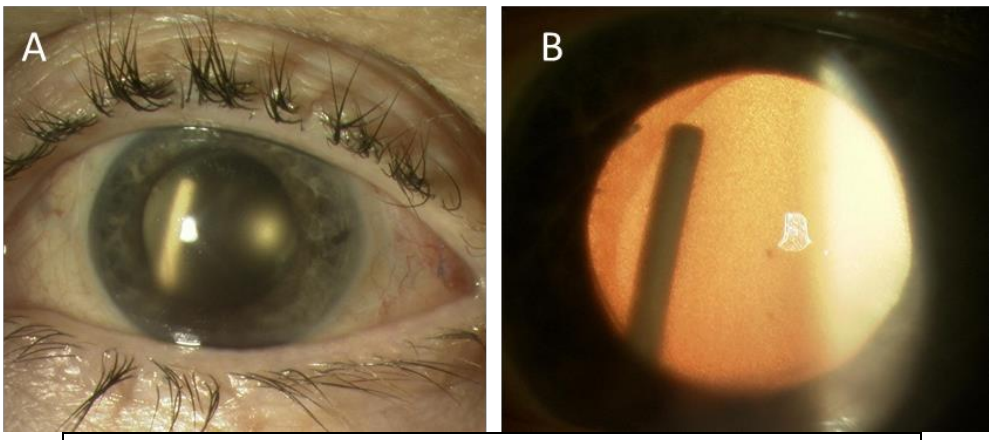


Figure 3.13 (A) AP colour photograph (B) retro-illuminated image showing the implant is trapped behind the IOL and anterior to the oil bubble. The patient reported that the 'white line' she had noticed in the pupil had disappeared 10 days prior to her 4 week postoperative review.

Case 2:

A second implant was similarly noted to be trapped behind the crystalline lens at Day 10, where it remained until its eventual resorption at 3 months. This patient underwent uneventful cataract surgery, at month three with no obvious adverse effects noted on the adjacent posterior capsule. There was gradual increase in nuclear sclerosis of the lens but no evidence clinically of increased localised lens opacity in proximity to where the implant had been anomalously situated.

Figure 3.14: Retro-lenticular Implant

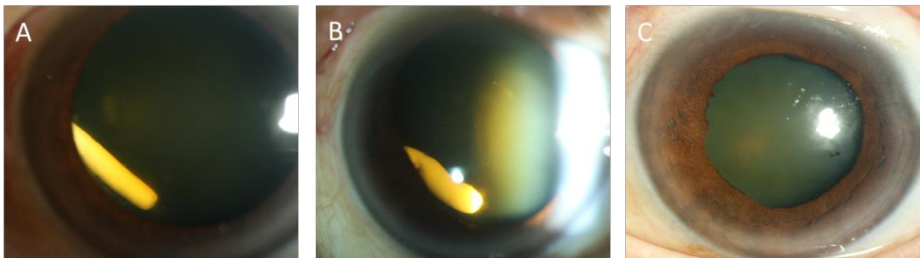


Figure 3.14 shows the sequence of AP photographs from Day 10(A) Week 4 (B) and Month 3(C) post implant injection. The implant can be seen to change morphology and shorten until its eventual resorption by month 3.

Considering the remaining sixty six implants; at day one approximately one half (47.1%) (n=32) of implants were positioned in the vitreous base (zone 3), with the remaining implants near equally divided between zones 1 and 2, (n=18 and n=16, respectively). No implants situated in the vitreous base at day one changed location throughout the follow up period.

3.2.6.4.3.3.2 Zone 1 Implants at Day 1

Considering the eighteen implants which were situated within the posterior pole (zone 1) at day one; by day ten, eight of these implants had spontaneously relocated to the vitreous base, with all but three of the remainder in zone 3 by day 30. This suggests that the overall incidence of trapped premacular implants in oil-filled eyes is 4.41%.

Interestingly, one of the eighteen implants was noted to 'travel' from zone 1 at day 1, to zone 2 at day 10 and eventually relocate to zone 3 by day 30. The three implants which remained 'trapped' at the posterior pole will be discussed further in due course.

Figure 3.15: Premacular dexamethasone implant

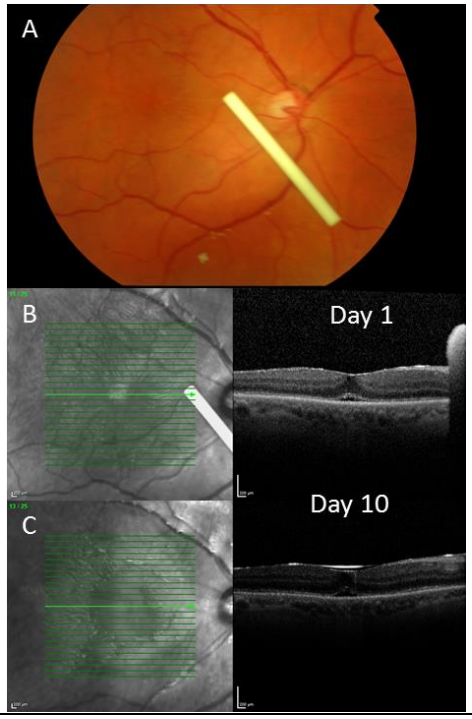


Figure 3.15 (A) colour fundus photograph of premacular dexamethasone implant at day 1. (B) Corresponding SD-OCT scan shows hyper-reflective signal from implant edge of image) and masking of reflectance of deeper tissue (C) by day 10 the implant has spontaneously relocated to the vitreous base, without any observed adverse effect

3.2.6.4.3.3.3 Zone 2 Implants at Day 1

Of the sixteen implants which were located outside the posterior pole, but within the equator (zone 2) the majority (n=10) had spontaneously relocated to zone 3 by day 10, with four further implants moving by day 30. Two implants remained 'trapped' in zone 2 throughout the follow up period. One of these implants became trapped anterior to the edge of a retinectomy against bare retinal pigment epithelium, without any adverse effect on outcome. The second remained 'caught' superior to the arcade (Figure 3.16 and Figure 3.17) again without any observed adverse effect on the adjacent tissue clinically.

Figure 3.16: An Implant Trapped in Zone 2

Figure 3.16(A) Fundus photograph showing the dexamethasone implant trapped in zone 2. (B) the implant is changing morphology as it degrades (C) fragmentation of the implant by month 3

Figure 3.17: SD-OCT image of Trapped Implant in Zone 2

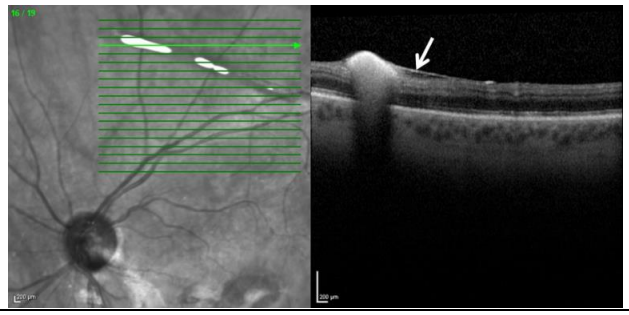


Figure 3.17 SD-OCT image of degraded implant at month 3 post injection. White arrow indicates hyper-reflective signal which may be the posterior meniscus of the oil bubble, or a fine epiretinal membrane.

3.2.6.4.3.4 Trapped Zone 1 Implants

The three patients in whom the implant remained trapped in zone 1 throughout the follow up period will be discussed in further detail herein.

Case 1

A 59 year-old Caucasian male at day 1 post study vitrectomy the dexamethasone implant was noted to be situated on the posterior pole with no obvious adverse effect. The eye was densely amblyopic with a posterior staphyloma and marked myopic macular atrophy. The implant remained visibly in situ at the day 10 and week 6 postoperative reviews. Figure 3.18. By month 3 the implant showed signs of dissolution with fragmentation into three segments. SD – OCT over the implant showed a transmission defect, and masking of the underlying reflectivity of the retina deep to it. No adverse effect on the adjacent retinal tissue was noted. Oil was retained long-term due to the high risk of retinal re-detachment.

Figure 3.18: Implant trapped in Zone 1 (Case 1)

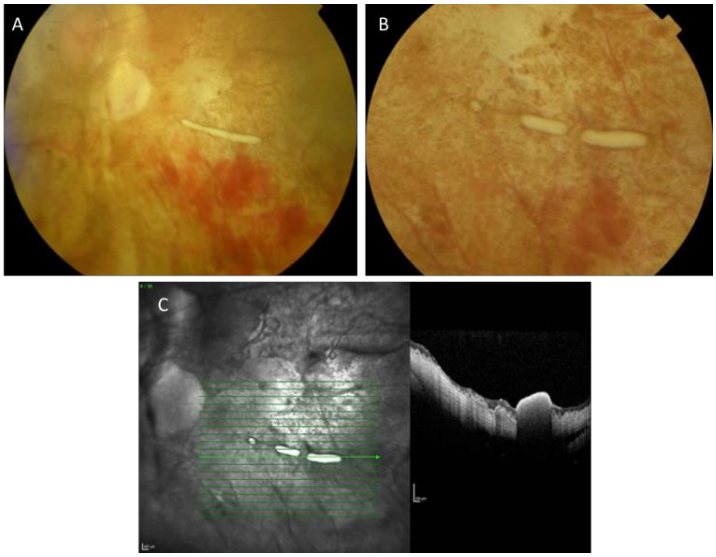


Figure 3.18 (A) 6 weeks and (B) 3 months post injection, the implant can be seen to degrade and fragment over the atrophic retinal tissue. (C) SD –OCT shows hyper-reflective signal from implant and transmission defect 'masking' reflectance from deeper tissue

Case 2

A 68 year old Caucasian male underwent a 3PPPV, peel of posterior membranes with the aid of perfluorocarbon (PFCL) liquid , a 270 degree retinectomy and silicone oil tamponade for a recurrent RD with severe PVR (Grade CP 12 [16]. At Day 1 the implant was positioned in Zone 1 and imaged at day 10 trapped behind the oil meniscus and adjacent to a retained subretinal bubble of PFCL. Oil was retained due to the high risk of recurrent RD associated with its removal.

Figure 3.19: Composite SD-OCT of trapped premacular implant

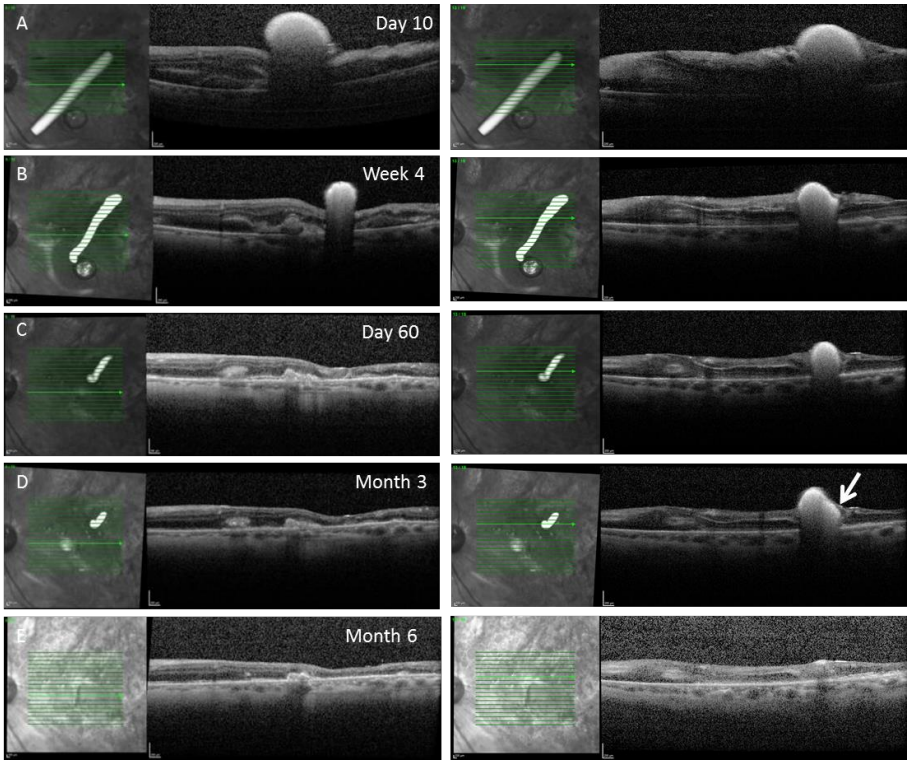


Figure 3.19 Left column show sequence through fovea and right sequence include implant until dispersion by 6 months. Note hyper-reflectance adjacent to the superior border of the implant edge which may represent local epiretinal membrane formation (white arrow). Also note left sequence highlights partial restoration of outer retinal architecture over 6 months.

Case 3

A 66 year old Caucasian male underwent his study PPV with ERM/ILM peel for a recurrent PVR (CP3). A large posterior break was adjacent to the macular hole which was treated intraoperatively. At day 10, the inferior edge of the trapped implant was situated over the treated break site (Figure 3.20). By day 30, an inferior macular branch vein occlusion was noted. The event was reported to the sponsor and the MHRA notified via the yellow card system. Combined removal of oil, PRP was performed with removal of the adherent trapped implant/ERM complex intraoperatively.

Figure 3.20: Composite trapped premacular implant at Day 10

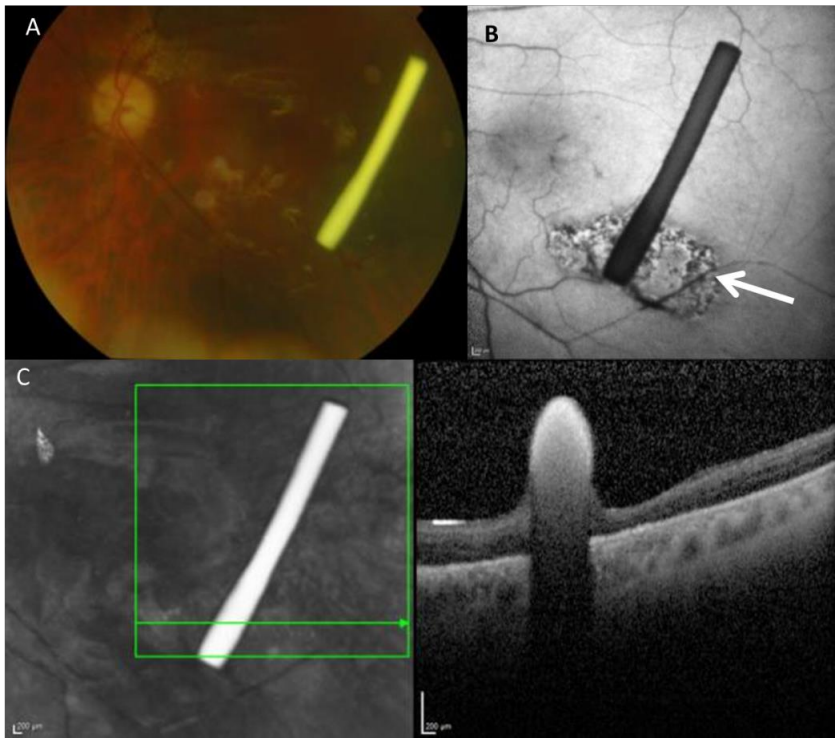


Figure 3.20 displays trapped implant Day 10 (A) coloured fundus photograph (B) autofluorescent image showing hypoflourescence at site of previous retinal break and surrounding inferior third of implant (C) SD OCT image . An inferior macular branch vein occlusion was noted at day 30. At the time of oil removal, the implant was firmly adherent to retina with associated epiretinal membrane

3.2.6.4.4 Discussion of implant position; nested study 2

Although, the appearance of the implant on the posterior pole may initially appear alarming, our series suggests that this is in fact a common event and occurred in over one quarter of cases (18 of 68). The overall incidence of trapped premacular implants was 4.4% (n=3 of 68).

Most implants (approximately 50%) were observed in the vitreous base by day one, and no change in position was noted thereafter.

Epiretinal membrane was noted in 2 of the 3 trapped premacular implants. Attributing causality to ERM formation is difficult in this population as membrane formation is a manifestation of the disease process itself. However, its presence may offer some insight into the negative findings relating to anatomical outcomes in this trial.

The concentration of dexamethasone may be expected to be higher, locally, surrounding the trapped implant. The formation of local ERM may indicate that the slow release preparation has insufficient therapeutic activity to modify the vitreoretinal scarring response. It is also possible that the release profile of the preparation was affected and thereby altering the pharmacokinetics.

Conversely, it is possible that the severity and extent of ERM may have actually been attenuated by the implants presence. The alternative sequelae of redetachment under oil may have ensued in its absence.

A BRVO was noted in case 3 which may have been caused by the mechanical obstruction of a macular branch vein. Again, attributing causality is difficult as ocular vascular occlusions occurred in other eyes in the study in both groups.

This nested study provided interesting results of the behaviour of the implant in this atypical environment. The findings associated with the trapped IMPs may offer insight into the therapeutic activity of the preparation on the PVR process.

3.2.7 Discussion

This is the first randomised clinical trial to investigate the use of a sustained release corticosteroid preparation as an adjunct in the treatment of established proliferative vitreoretinopathy.

3.2.7.1 *Recruitment and Retention*

Recruitment rates were initially well ahead of target but reduced from month nine until month 13. This reflects the period with the maximum number of active study participants. Since my role involved both recruitment and the clinical management of all 140 study participants throughout the trial, a reduction in recruitment may have been an inevitable consequence. This could be anticipated in future studies and strategies to cover this 'bulge' in work load implemented accordingly.

Nevertheless, as participants completed the study the 'resource strain' may have been alleviated, allowing us the opportunity to complete recruitment close to target.

We observed a high eligibility to enrolment ratio which again may be in part explained by the pragmatic trial design. Study follow-up visits reflected clinical practice thereby nullifying the 'burden' of additional visits which some studies adopt. Furthermore, the natural history of standard care carries a poor prognosis. Patients who were eligible for the study had frequently suffered multiple failed procedures, as shown in section 2.4.5.4, with two thirds of patients having had at least one previous operation. Offering the opportunity to receive a potentially beneficial intervention with a well-documented side effect profile was usually received positively.

Study retention was also favourable with primary outcome data available for 138 out 140 participants.

3.2.7.2 *Anatomical Outcome Discussion*

For the purposes of this thesis, anatomical outcomes will refer to surgical success (primary outcomes and retinal reattachment rates), overt PVR recurrence and epiretinal membrane formation. Visual outcomes and cystoid macular oedema will be discussed in parallel, due to their close relationship in this disease population.

3.2.7.2.1 Primary Anatomical Outcome

The primary outcome was a comparison between the proportion of eyes in which stable retinal reattachment was achieved in the absence of internal tamponade without additional vitreoretinal intervention. (i.e. primary success with oil removal by month six post study vitrectomy). Reported anatomical outcomes in the surgery for established PVR vary considerably.

We found no difference in this outcome measure. Approximately one half of patients achieved primary success in both groups (49.3% vs 43.3%, adjunct vs control), which is similar to previously published rates in RCTs adopting a comparable primary outcome measure [98, 122].

In a study comparing the effect of 4mg of intravitreal triamcinolone, Ahmadieh *et al* [83] published an overall primary success rate of 81.3% in eyes with Grade C PVR undergoing vitrectomy surgery with an encircling scleral buckle. They observed no difference in primary or secondary outcomes between the adjunct and control arms. This rate of retinal attachment is markedly higher than other reported series, and has yet to be replicated. Cheema *et al* reported an eventual anatomical success rate of 87.5% (21 of 24eyes) at 12 months [78]. Drawing direct comparisons is difficult as their study was retrospective and non-comparative. Furthermore, disparate definitions of success may explain their high anatomical success rate. Eyes with silicone oil *in* situ were included in their definition of success with only 4 of the 21

'successful cases' undergoing oil removal. We observed an eventual anatomical success rate of 66.7% vs 69.6% at 6 months, in the adjunct and control group respectively. However, if eyes with retained oil are included, our 'success' rate increases to 93% (64 of 69) in both groups.

The Silicone Oil Study reported visual and anatomical outcomes on eyes with established PVR. The first report compared silicone oil with sulphur hexafluoride (SF_6) in eyes with primary PVR and found the former superior [251]. Macula attachment was more common in eyes managed with SO (80%) compared to SF_6 (60%). In the second report, SO was compared to perfluoropropane (C_3F_8) in both primary and secondary PVR. Final anatomical rates were similar (73% vs 64%, respectively). Criteria for success included eyes with retained silicone oil. Our study outcomes compare favourably to the Silicone Oil Study Report.

As discussed in Chapter 1, the current grading of PVR has served to standardise terminology, but remains limited in some respects. The extent of PVR is classified in only one circumferential axis, and thus linear bands of membrane (particularly) subretinal, may be apportioned the same PVR grade as extensive posterior retinal sheets of membrane. This may offer an explanation as to why the sensitivity analysis adjusting for baseline PVR grade failed to show differences in outcome in either group.

The heterogeneous nature of the population may have limited our ability to detect small differences between the two groups. As the study design was pragmatic and aimed to reflect clinical practice this is an inevitable consequence.

The study was powered to improve failure rates from 49% to 24%. The primary outcome measure was set to reflect a clinically valid treatment effect. This was not achieved and hence a shift of focus towards potential treatment effects in secondary outcome measures will be discussed.

3.2.7.2.2 Secondary Anatomical Outcome Findings

If we consider secondary outcomes indicative of the effect of the IMP on the proliferative component of the PVR process, we found no discernible differences between the two groups. A comparable proportion of patients achieved complete or posterior retinal reattachment and the proportion of eyes with a tractional RD or macular pucker was also similar between the two study groups. Furthermore, rates of overt PVR recurrence were similar across both groups (58.0% vs 59.4%, adjunct vs control). Our lack of treatment effect on the anatomical outcomes in this study reflect the findings of Kuo *et al* [72] in their experimental study of the same preparation. However, the significant reduction in inflammatory cytokine expression may explain our proposed positive treatment effect in reduction in cystoid macular oedema rates. This will be discussed further in due course.

We did note that fewer patients in the adjunct group (n=3) required two or more operations to achieve attachment compared to the control group (n=11). However, as this was not investigated as a secondary outcome and numbers are small, we did not test for statistical significance, and caution must therefore be advised when interpreting this difference.

Epiretinal membrane formation rates were comparable across both groups. Observations from the second nested study (section 3.2.6.4) may have provided insight into the local therapeutic action of the IMP when trapped. OCT guided visualisation of the sequelae longitudinally, revealed progressive ERM formation adjacent to and involving the trapped implant. This may suggest that the IMP is ineffective at modifying the local scarring response or may simply be an indication that the balance of activity remained in favour of the PVR process.

Taking these secondary findings in conjunction with results of the primary outcome measure, we can conclude that a slow –release dexamethasone implant does not improve the anatomical success rates in eyes with established PVR.

3.2.7.3 *Visual Outcomes*

As visual acuity was investigated as a secondary outcome, it is important to acknowledge that any observed estimates of treatment effect may be imprecise. Where statistical comparisons have been made, these findings are being reported as exploratory.

Previous studies investigating the effects of pharmacological adjuncts in eyes with established PVR have also primarily investigated anatomical outcomes at either 6 or 12 months post-surgery. However, reported secondary visual outcomes range considerably from 1.2 logMAR to 2.69 logMAR [83, 98, 122]. The visual outcomes in both groups in our cohort compare favourably with the aforementioned previously published data. Chang *et al* reported on outcomes in eyes with primary PVR [122] whereas Charteris *et al* [98] included eyes with both primary and secondary PVR, and thus is likely more akin to our dataset. Ahmadieh *et al [83]*reported a best corrected visual acuity of 1.2 LogMAR in eyes receiving IVTA compared to 1.4LogMAR in control eyes at 6 months.

The mean VA in the adjunct group was 38.3 ETDRS letters (standard deviation 23.7) compared to 40.2 letters (standard deviation 21.1) in the control group. This equates to LogMAR VAs of 0.96 and 0.90, and approximates to a Snellen VA of 20/160. Similarly, the proportion of eyes achieving a visual acuity ≥ 55 ETDRS letters (> 20/80) was also comparable (30.4% vs 24.6%, adjunct vs control)

The overall favourable visual outcome in our cohort as a whole (as there is little difference between the groups) may be indicative of a general improvement in surgical technique but a paucity of corroboratory contemporary data limits our ability to confidently draw this conclusion.

If we consider the change in vision from baseline we did not observe any difference between the two groups gaining 10,20,30,40 or 50 letters. However, when considering the comparison between the proportion of eyes gaining 60 letters or more, the observed difference is more apparent. This comparison may show a trend towards a treatment effect of the adjunct.

On exploratory sub analysis of eyes without limited visual potential at baseline, we found that the vision was consistently higher in the adjunct group compared to the control group until month 6 when the results become comparable. In fact the mean difference in 7 letters at Day 60 (45 letters vs 38 letters, adjunct vs control) achieved statistical significance. Although this difference may be of marginal clinical significance, it is an interesting finding.

Further discussion regarding visual outcomes in relation to cystoid macular oedema will follow the discussion regarding macular findings.

3.2.7.4 *Macular Findings*

As discussed in 3.1.2.2, the slow release dexamethasone implant is licensed for use in the treatment of macular oedema secondary to retinal vein occlusion and diabetes. Its therapeutic effect in vitrectomised eyes has also been discussed.

Estimates of the incidence of postoperative cystoid macular oedema following retinal detachment repair are difficult to reliably interpret from the literature. Previous estimates range from <1% in eyes without PVR [11] to 66% in PVR eyes with limited visual recovery [252]. Clinical assessment of the presence of CMO is likely to underestimate its incidence. OCT has a higher sensitivity of detecting subtle anatomical macular changes which may not be apparent on fundal examination. In a recent study of 587 eyes with primary RD treated with either small gauge vitrectomy alone (n=461) or combined vitrectomy and scleral buckle (n= 126), the OCT guided incidence of CMO was 31.2% vs 48.4% (n= 144 and 61, respectively).[253]

The pathophysiology of CMO is complex involving a variety of processes. Blood-retinal barrier breakdown and increased vascular permeability underlie its development. Inflammatory cytokines and growth factors (interleukin (IL-1), tumour necrosis factor (TNF)-alpha, and vascular endothelial growth factor (VEGF)) are released by activated leucocytes which have migrated into the extracellular space and further increase vascular permeability. The inflammatory process is thereby amplified. Expansion of extracellular spaces with resultant fluid accumulation, overwhelm homeostatic fluid balance mechanisms thereby leading to retinal thickening and commonly visual loss [254].

It is reasonable to presume that the incidence of CMO may be higher in eyes with proliferative vitreoretinopathy as the circulating levels of inflammatory cytokines in the vitreous and retinal microenvironment are high [255-258]. Benson *et al[252]* .

reported a 66% incidence of CMO in 35 eyes with proliferative vitreoretinopathy 3 months after successful retinal reattachment surgery. This figure is comparable to the observed rate of 67.1% in our control group.

It is accepted that CMO is a cause of visual loss. Despite finding significant differences in rates of CMO at 6 months we did not find any difference in visual acuity at the same time-point. When adjusting for eyes with limited visual potential at baseline, the similarity in visual outcome was unaffected.

Furthermore, if we take CMO as the independent variable, and subsequently compare the visual outcome in eyes with and without this pathology, we *do* observe a meaningful difference (3.2.5.9.1.1). This difference is apparent in the cohort as a whole and also when sub-analysed by treatment group.

Therefore the following conclusions can be made from the findings of our study. CMO is associated with poorer vision in eyes with PVR. A reduced rate of CMO at 6 months is observed in eyes treated with a slow release dexamethasone implant. One may therefore question why we did not observe a treatment effect of the adjunct.

Firstly, since the study was powered to detect a difference in anatomical outcome, it is likely that we have insufficient power to detect a difference in visual acuity between the two groups.

If we refer to Figure 3.7, we note that the adjusted mean VA at Day 60 is significantly higher in the adjunct group (45 letters) compared to the control group (38 letters). As this study visit was scheduled primarily to monitor IOP, an OCT scan was not routinely performed and we did not collect data on the presence of CMO at this time point. Pharmacokinetic and clinical studies (section 3.1.2.1), consistently describe a maximal treatment effect at 2 months post injection, which support our findings.

Secondly, the strength of the association between CMO and vision may not have been sufficient for us to observe an effect in this study.

Finally, it is reasonable to assume that there are factors other than CMO which contribute to visual loss in eyes with PVR and this warrants further investigation. This will be discussed further in Chapter4.

It would be interesting to determine whether the difference in rate of cystoid macular oedema is sustained during the second 6 month period or whether the observed effects are transient. As the intraocular concentration of corticosteroid decreases and the effect of the implant diminishes, the rates of CMO may return to similar levels between the two treatment groups.

3.2.7.5 *Quality of Life Analysis*

We did not observe any differences in either of the two quality of life (QAL) parameters which were used in the study. Further analysis of the subgroups may suggest differences in sub groups of the QAL questionnaires, but this lies outside the scope of this thesis.

3.2.7.6 *Intraocular Pressure Findings*

3.2.7.6.1 Elevated IOP following vitrectomy with Silicone Oil

Estimates of the incidence of raised intraocular pressure (IOP) after pars plana vitrectomy with silicone oil are difficult to reliably interpret from the literature. Disparate definitions of diagnostic criteria for raised intraocular pressure and glaucoma contribute to the wide variation in reported rates to date [259]. Additionally, the incidence of ocular hypertension and/or glaucoma has often been further sub-categorised in relation to the timing of silicone oil injection. Thus the researcher is not only required to infer rates from inconsistently defined outcome variables, but also must attempt to draw comparisons from conclusions drawn from temporally disparate events.

The earliest comprehensive report of IOP-related complications following the injection of silicone oil was described in 1993 in the Silicone Oil Study Report 4[260]. In 241 eyes with established (Grade C) PVR randomised to either intraocular perfluoropropane gas or silicone oil tamponade , the incidence of chronically elevated IOP was significantly higher in those treated with oil (2% vs 8%, respectively). Criteria for the aforementioned complication included two consecutive or three independent postoperative IOP measurements of >25mmHg using applanation tonometry. Six years later, Henderer *et al* [261] reported a 21% incidence of prolonged elevated IOP in 532 eyes undergoing vitrectomy surgery with silicone oil. Prolonged elevated IOP was defined as an IOP requiring operative intervention at any time-point, or an IOP of greater than 25mmHg at or beyond month 6 post oil injection. Concurrently, Honavar *et al [262]* published findings of a retrospective study of 150 eyes undergoing vitrectomy surgery with silicone oil injection for complicated retinal detachments due to PVR, proliferative diabetic retinopathy and trauma. Glaucoma was defined as an IOP of ≥24mmHg, which was ≥10mmHg higher than baseline, and sustained for ≥ 6 weeks. Glaucoma was found to occur in 40% (n=60) of eyes and directly attributable to silicone oil in 70% of cases. Although approximately three quarters of eyes were

controlled with medication alone (30%), medication and removal of oil (25%) and filtration/aqueous shunt surgery/cycloablative procedures (17%), refractory 'glaucoma' remained in 28% of eyes. Emulsification of silicone oil was found to be predictive of poorer IOP control.

However, Jonas *et al [263]* subsequently reported a 7.1% incidence of glaucomatous optic neuropathy in 198 eyes treated with 5000centistoke silicone oil. This was in spite of finding a 20% rate of IOP rise (defined as at least one episode of >21mmHg) and 20.2% oil emulsification rate. Contrasting findings may be explained in part by the disparate diagnostic criteria, but also the shorter follow up period in the latter.

More recent studies report incidences of postoperative elevated IOP ranging from 11% to 30% [264-266], although a trend towards adopting a lower threshold of 21mmHg is apparent.

3.2.7.6.2 Corticosteroids and elevated IOP

Aside from the reported increased risk of IOP rise secondary to silicone oil injection, the potential steroid-induced response from the dexamethasone implant may confer an additional risk of IOP elevation.

The mechanism of steroid induced IOP rise is incompletely understood. It can occur following a variety of modes of administration, although it is more commonly associated with the use of potent topical corticosteroid use such as prednisolone and dexamethasone. [267] It was first reported in 1950 by Mclean *et al [268]* following the systemic administration of adrenocorticotrophin hormone (ACTH), and later with local cortisone injection[269]. The physiological diurnal variations in IOP correlate closely with plasma cortisol levels, and this variation is lost following adrenal gland removal [270].

It is generally accepted that the mechanism of induced rise in IOP is at the level of the trabecular meshwork and believed that corticosteroids induce an outflow obstruction through an inhibition of extracellular matrix degradation and accumulation of muccopolysaccarides [271-274].

3.2.7.6.3 Slow-release dexamethasone and elevated IOP

Published clinical studies investigating the use of Ozurdex have defined post injection elevated intraocular pressure as an IOP ≥ 25mmHg. The reported incidence of raised IOP following the injection of Ozurdex has ranged from 24.0% in non-vitrectomised eyes with venous occlusion [221] to 36% in vitrectomised eyes with diabetic macular oedema [230]. In our study, the incidence of at least one episode of IOP rise by month 6 was 45.7% (n=32) in the adjunct group compared to 31.4% (n=22) in the control group.

As mentioned previously, it is difficult to draw direct comparisons between previously published data on Ozurdex-induced elevated IOP and our group. Aside from variations in diagnostic criteria, there are multiple additional potential factors which may contribute to ocular hypertension in this complex patient population i.e. post-pars plana vitrectomy alone, silicone oil tamponade, postoperative uveitis and the concurrent use of topical corticosteroids. Haller *et al's* [221] incidence of 24% IOP rise can be quite confidently attributed to a steroid response induced by the administration of Ozurdex, whereas we cannot directly draw that conclusion. If we consider our control group, the observed incidence in IOP rise of 31.4% is comparable to previous reports.

The mean intraocular pressure peaked at day 10 post study vitrectomy in both groups and was not found to be statistically significant. However, mean IOP was significantly higher in the adjunct group at Day 60 post study vitrectomy and Day 60 post removal of oil. As the implant was injected on two occasions in the adjunct group (i.e. study vitrectomy, and removal of oil procedure) it is highly likely that this difference can be confidently attributed to an Ozurdex-induced steroid response. This is further corroborated by previous studies in which the maximum IOP rise occurred at a similar time-point [14, 221, 230]. Interestingly this difference is most marked at Day 60 post ROSO when there is no intraocular tamponade, perhaps suggesting that the presence

of silicone oil affects the pharmacokinetic profile of the release of dexamethasone and thereby may have attenuated any steroid response.

Although, in total, there were more IOP adverse events in the adjunct group (81 events) compared to control group (70 events), and a larger proportion of patients suffered at least one IOP rise in the adjunct group (45.7%) compared to the standard group (31.4%), the majority were classified as mild events (i.e. IOP >25mmHg but <35mmHg). Interestingly, the proportion of mild rises in IOP was 74.1% (n=60 of 81 events) in the adjunct group compared to 60% (42 of 70 events). The proportion of moderate or severe IOP rises were lower in the adjunct group as follows; 17 of the 81 events (21.0%) were classified as moderate compared to 23 of 70 events (32.9%) in the control group (Chi squared 2.11, p=0.143); 4 of 81 events (4.9%) in the adjunct group were classified as severe compared to 5 of 70 (7.1%) in the control group.

One might have expected a greater difference in the incidence of IOP rises in the treatment group compared to the standard group. However, the interplay of factors which may either induce an elevated IOP, mitigate against a pressure rise or even predispose to hypotony makes interpretation of these results complex. Factors which may induce an IOP rise may at least in part be offset by surgical interventions that predispose to hypotony. A large proportion of patients underwent a retinectomy either prior to entering into the trial, during their primary study vitrectomy or at reoperations following recurrent retinal detachment. These eyes, by consequence, have an increased surface area of exposed retinal pigment epithelium and therefore a subsequent increase in aqueous outflow via the uveoscleral path through the choroid. 49 of the 70 eyes (70.0%) in the control group had undergone a retinectomy and 45 of 70(64.2%) in the adjunct group. Furthermore, severe anterior PVR can reduce aqueous production through cyclitic membrane formation and secondary ciliary body detachment [275] Although no formal imaging assessment of ciliary body status was performed for the purposes of this study, it is likely that this may help to explain why silicone oil was retained in a proportion of eyes where there was insufficient IOP to allow its removal.

The intraocular pressure may either be reduced, unchanged or elevated in the inflamed eye. The former may be secondary to reduced outflow from a secondary trabeculitis and the latter owing to a reduced aqueous production as the secretory ciliary epithelium function may be impaired [275].

We noted a lower incidence of postoperative uveitis in the adjunct group (10 patients 14.3%) compared to the control group 23 patients (32.9%). Relating this to IOP variations may be complicated, but again may be indicative of a treatment effect of the adjunct.

3.2.7.7 *Hypotony*

Rates of hypotony (defined as an IOP <6mmHg) were comparable across the two groups with 14 patients (20.0%) suffering at least one episode in the adjunct group compared to 17 patients (24.3%) in the control group. Just under one half (48%) of all episodes of hypotony were recorded at Day 1 post removal of oil.

Hypotony post ROSO is a widely reported event. Kim *et al [276]* retrospectively reviewed 89 consecutive eyes treated with silicone oil following vitrectomy for complicated retinal detachment. They reported an incidence of 39.3% of transient hypotony post silicone oil removal. A binary logistic regression model found axial length to be a predictor of postoperative hypotony with an Odds Ratio of 1.385 (p=0.023). Interestingly, preoperative IOP, severity of PVR, number and type of previous operations and duration of tamponade were not found to be predictive of post ROSO hypotony. In our study, silicone oil was removed in 111 of 140 eyes. Postoperative hypotony occurred in 27 of 111 eyes (24.3%) of eyes, but recovered in the majority.

3.2.8 Conclusion

This study suggests that adjunctive slow-release dexamethasone does not improve anatomical outcomes in eyes with PVR. We observed a reduction in CMO in the treatment group. Exploratory sub-analysis may suggest a corresponding improvement in vision at the point of peak intraocular corticosteroid concentration at Day 60.

The primary research paper is currently under review by Moorfields Research Management Committee, awaiting approval for submission for publication.

3.2.9 Lessons Learned

3.2.9.1 *MHRA Inspection*

On December 17th 2013, the Ozurdex in PVR Study team received electronic confirmation that Moorfields Eye Hospital as Sponsor of non-commercial clinical trials of investigational medicinal products (CTIMPs) would undergo a routine GCP inspection by the MHRA. Four CTIMPs were chosen to undergo study specific inspections, three of which were investigating the same medicinal product, Ozurdex ®. All three of these trials were actively recruiting participants. The inspection was scheduled to take place between 11th and 13th of March 2014, allowing a three month period of preparation.

3.2.9.1.1 MHRA regulatory process; inspections

Current clinical trial regulations make MHRA inspection a statutory requirement (2001/20/EC Article 15 (1) (2) and 2005/28/EC Article 21-30.) This mandates the inspection of trial sites, manufacturing sites of investigational medicinal products (IMPs), laboratories used for sample analyses (where appropriate) and Sponsor premises. The aim is to monitor compliance with the provision of Good Clinical Practice (GCP) and Good Manufacturing Practice (GMP).

More specifically the 2001/20/EC European Union Clinical Trials Directive defines an Inspection as follows:

'*An act by a Competent Authority (CA) of conducting an official review of documents, facilities, records, quality assurance arrangements and any other resources that are deemed by the CA to be related to the clinical trial and that may be located at the site of the trial, at the sponsor's and or contract research organisations (CRO) facilities, or at other establishments which the CA sees fit to inspect*'

There are three types of Inspection:

- **Routine inspections**: *inspections of the systems and procedures used to conduct clinical research in the UK*
- **Triggered inspections**: *ad hoc inspections triggered as a result of MHRA licensing requests or reports received on suspected violations of legislation relating to the conduct of clinical trials.*
- **Committee for Medicinal Products for Human Use (CHMP) requested inspections resulting from central MA submissions**: *The EMEA (The European Medicines Agency) co-ordinates these inspections, which are conducted by inspectors from the EU Member States.*

Routine inspections can be further subdivided into:

- *Systems Inspection* (either Sponsor or CRO) -in order to evaluate the quality assurance and quality control systems established by the organisation

- *Specific Clinical Trial Inspection* – in order to verify that the trial has been/is being conducted appropriately such that the data generated is documented and reported in compliance with a) the study protocol b) according to GCP principles and c) according to sponsor procedures

The MHRA provide a notice period of approximately 12 weeks to the organisation, and thereafter detail a list of requested information in a dossier. The MHRA must then be in receipt of the requested information within 28 days of notice being served. The MHRA may then confirm the date of the inspection and will provide an outline plan four weeks in advance of the upcoming inspection.

The draft inspection plan outlines the timetable of events and follows a relatively standard format. This includes an opening meeting during which the scope of the inspection is set and the inspectors are introduced. The plan also highlights the processes which are to be assessed by session allocation, and the relevant personnel who are invited to interview. Finally a 'close out' meeting is held during which the preliminary findings of the inspection are discussed.

This process was followed for the routine sponsor inspection at Moorfields Eye Hospital in March 2014. The inspection plan confirmed that the Ozurdex in PVR study was selected for a trial-specific inspection and that key members of the study team were invited to interview. As the research fellow coordinating and running the trial, I was invited to interview by the MHRA and sat *in loco* PI in his absence. I therefore led the study team both through the process of inspection preparation and during the formal structured interview. An outline of my role during this process will be discussed herein:

3.2.9.2 *Inspection Preparation*

The intricacies and minutia which constituted twelve weeks of intense preparation for this major event are well beyond the scope of this thesis. However, key aspects which were identified during the preparation process will be highlighted. They are valuable learning points which I will take forward in my continued development as a clinical trialist and have already incorporated these into projects which have grown out of this research.

3.2.9.2.1 In Search of the Perfect Protocol

All clinical trials begin with the development of a clinical protocol. This document describes in detail how the trial will be conducted and states the rationale, objective, design, methodology, statistical considerations and organisation of the study. It should ensure the safety of trial subjects and protect the scientific integrity of the trial. There are a number of useful templates available and prior to the MHRA Inspection, the Sponsor (MEH) instituted a standard operating procedure (SOP) outlining the required processes during study setup. These included a protocol template to facilitate investigators in protocol development. These processes were not yet in place during the development of the Ozurdex in PVR Study protocol and thus the MHRA inspectors were unable to review our study against these SOPs. This will be discussed in further detail in due course.

By definition, in order to gain ethical approval and be granted an authorisation to conduct a clinical trial by the relevant regulatory authorities, the protocol must be written in advance of commencing the trial. During the active phase of the trial it may become necessary to modify the study design particularly if new safety information arises.

3.2.9.2.2 Protocol Deficiencies Identified in the Inspection Preparation

During the inspection preparation process, a review of adverse events was conducted in a trial management group meeting and it was noted that 'cataract' as an expected adverse event had not been recorded as having occurred in any patients at the point of review, some 18 months into the trial. As the research fellow clinically managing these patients I had confirmed that this was indeed the case. The pharmacovigilance officer had previously contacted the study team five months earlier informing us that a collective SUSAR had been submitted to the MHRA following the increased rate of cataract formation in eyes treated with Ozurdex. This was noted in a CTIMP using the same medicinal product but in a different patient population at the study site. Upon

receipt of this mail, a response was drafted on behalf of the study team stating that the team's definition of cataract as an adverse event was study-specific.

As cataract formation is an expected consequence of vitrectomy surgery and that cataract surgery at the time of oil removal is considered routine, the study team considered the unexpected rapid progression of cataract as an adverse event and that clarifying this as a minor amendment to protocol could be done should the sponsor require it. This was acknowledged by the pharmacovigilance officer and the study team did not receive any further instruction from the Sponsor.

Upon further review by the Sponsor's quality assurance (QA) advisors prior to the inspection, it was identified that the study team's definition of cataract as an adverse event was not explicitly stated in the protocol. Furthermore, cataract surgery requires an admission to hospital (albeit as a daycase admission) and therefore satisfies the criteria for seriousness. An adverse event is any untoward occurrence in a subject participating in the trial irrespective of the relationship to the IMP. Therefore, as a proportion of patients entered the trial without evidence of cataract at the baseline assessment and had subsequently undergone cataract surgery, according to the then working study protocol, these patients had by definition suffered serious adverse events (SAE). These SAEs had not been reported to the sponsor.

Upon discussion with the sponsor, it was agreed that a substantial amendment be submitted clarifying the definition of cataract in the context of the Ozurdex in PVR Study and a file note was created explaining the course of events which had led to the oversight.

3.2.9.3 *MHRA Inspection Findings*

Although the study team were commended on our efforts throughout the MHRA Inspection by both the Sponsor and the MHRA, reference to the lack of clarity in the protocol was raised as a significant finding. Whilst it was acknowledged by the Inspectors that the safety of patients in the trial had not been compromised, we had contravened regulation by effectively instituting a substantial protocol amendment without gaining regulatory authority approval.

A corrective and preventative action plan was submitted in response to the finding acknowledging the oversight and the introduction of a revised CTIMP protocol template to ensure that safety sections were addressed more concisely and included rationale for risk adapted recording and reporting of AEs for future trials.

The ASCOT study (refer section2.4.8) was one of the first clinical trials to follow the new protocol development process. This risk adapted approach was adopted in the writing of the study protocol and I shall continue to use the knowledge I gained throughout the inspection process in future RCTs.

4 Identification of prognostic factors for visual outcome

4.1 Background

The visual outcome in patients undergoing surgery for PVR is dependent on numerous factors. Amongst them, cystoid macular oedema is a key finding in eyes with limited recovery [252]. The presence of CMO may be considered a surrogate marker of inflammation. The study findings outlined in Chapter 3 suggested a lower rate of cystoid macular oedema at the primary end point in eyes treated with the corticosteroid implant. We also demonstrated that eyes with CMO see worse than those without.

Although CMO is associated with poorer vision, we did not observe a corresponding treatment effect in terms of visual outcome at this time point. We did, however, observe a more favourable visual outcome at two months post injection. As the study was not powered to detect this difference, the strength of the association of CMO and vision was not sufficient to observe this potential treatment effect, and additional factors may contribute to visual loss. These require further investigation.

4.2 Aims

In this Chapter, I aimed to use spectral domain -optical coherence tomography for two purposes. Firstly, I aimed to identify features on the OCT which may correlate to visual recovery following successful repair of RDs with PVR. These findings may serve to identify neuroprotective and regenerative targets for future studies.

Secondly, I aimed to assess the use of OCT as an objective measure of vitreous inflammation in eyes with PVR. This may serve to define clearer and achievable endpoints in PVR trials, where modifying the inflammatory process may be key to managing the condition.

4.3 Photoreceptors

The human eye contains two types of photoreceptors; rods and cones. They constitute the outermost layer of the neurosensory retina. There are approximately 115 million rods and 6.5 million cones in the eye. The density of each cell type varies in different regions of the retina, with cone density increasing towards to the macula where the fovea is exclusively cones (150 000 mm2). The peripheral retina is dominated by rods (30 000 per mm2) [3].

Rod photoreceptors sense contrast, motion and brightness, whereas cones are responsible for fine vision, spatial resolution and colour perception. Both cell types share common anatomical features, being long narrow cells with an inner and outer segment joined by a connecting stalk of modified cilium. The shape of their outer segments differ as their name suggests, with rods adopting a cylindrical shape compared to the conical outer segments of cones.

The nucleus of the cell body (ONL) is 'separated' from the inner segments by an external limiting membrane (ELM) and project axons through the outer plexiform layer to form synaptic terminals. Here they synapse with bipolar and interneurons. These cone and rode synaptic terminals are termed pedicles and spherules, respectively [3].

The cone photoreceptor inner and outer segment anatomy and physiology will be discussed in further detail due to its reported significance on central visual function after retinal detachment repair.

Three types of cone photoreceptor cells exist in humans and are referred to as blue, green and red according to their relative light wavelength sensitivity i.e. short, medium

and long wavelength, respectively. The conical outer segments are shorter than rods (6um at the base and 1.5um at the apex) and contain the visual pigments known as opsins. Cone opsins can be further divided in to photopsin I, II and III depending on the aforementioned cell type in which they are found. These pigments are arranged in discs which are stacked throughout the length of the outer segments and communicate freely with the interphotreceptor proteoglycan matrix. The villous melanin-containing apical processes of the RPE surround the outer segments.

The cone inner segments consist of an inner half known as the myoid region and an outer ellipsoid region. The inner segments are metabolically and synthetically active. The myoid region contains numerous organelles including smooth endoplasmic reticulum, Golgi bodies, ribosomes, microtubules and glycogen. The ellipsoid region is densely packed with elongated mitochondria arranged in parallel. The ellipsoid region communicates with the outer segment via a modified cilium which serves as a conduit for metabolites and lipids [3].

4.4 Phototransduction

The perception of light is mediated by opsins, which are found in the photoreceptor outer segments. These G proteins (opsins) are bound to a vitamin A-derived chromophore, 11 cis-retinal. Upon absorption of a photon of light, the phototransduction cascade is commenced by the *cis-trans* isomerisation of these transmembrane proteins. The eventual hydrolysis of cGMP following the transducin-induced activation of phosphodiesterase, results in a 100-fold amplification at every stage. Closure of Na^+ channels is achieved by falling levels of cGAMP with resultant photoreceptor hyperpolarisation [277]. This results in the subsequent depolarisation of ON bi-polar cells and progression of the neuronal impulse along the visual pathway.

4.5 Visualising Photoreceptors – Optical Coherence Tomography (OCT)

Relatively recent advances in imaging modalities have served to allow us to visualise the retinal microstructure *in vivo.* Comparisons between normal and abnormal have further helped us to understand the pathological basis of some ocular disease and relate these findings to clinical outcomes.

Optical Coherence Tomography (OCT) is a non-invasive technique which uses light in the near-infrared spectrum to create high resolution cross sectional and 3 dimensional images of the retina which are thought to correlate well with retinal histology.

4.5.1 Optical Coherence Tomography – principle

OCT uses light of 810nm wavelength emitted from a super luminescent diode which is split by a partially reflective mirror into a measuring beam and a reference beam. The measuring beam is directed into the eye and reflected to variable extents by succeeding ocular interfaces (e.g. retinal layers, RPE and choroid). The reference beam is directed to a reference mirror whose position is adjusted to synchronise with the returning measuring beam which has reflected from the retinal surface. This results in constructive interference. Deeper structures reflect the measuring beam out of phase with the reference beam and produce variable degrees of destructive interference. This interference is measured by a photodetector and processed into a signal which can be displayed as an image. The signal amplitude determines the brightness of the image with highly reflective surfaces seen as brighter lines.

Figure 4.1: Schematic Diagram of OCT Principle

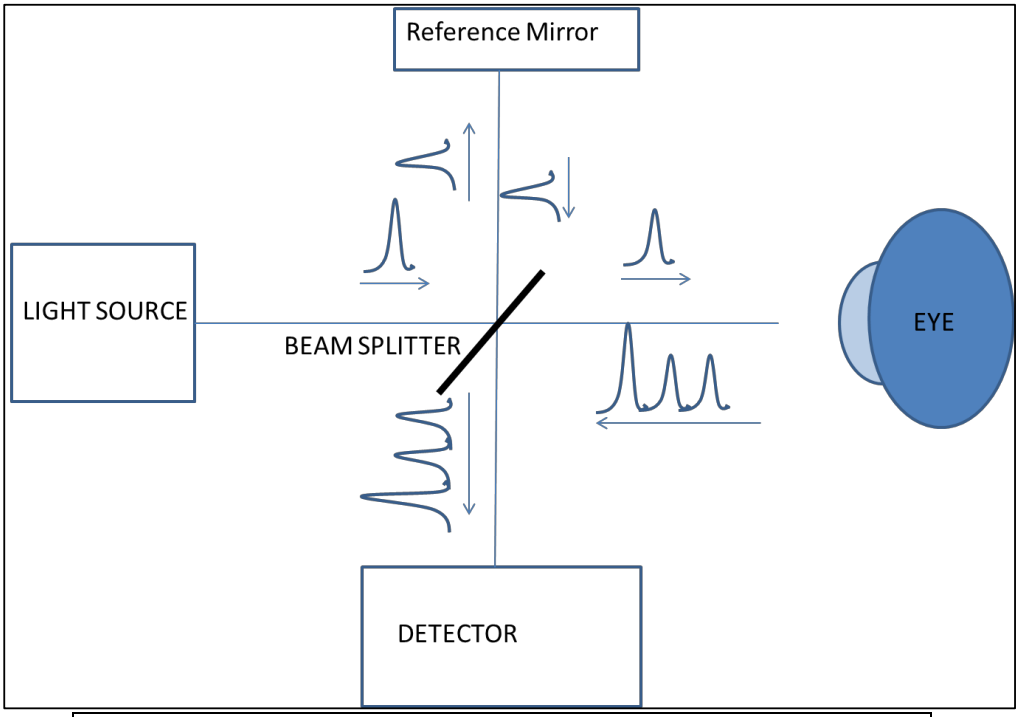


Figure 4.1 Schematic diagram displaying principle of OCT. 810nm light emitted from super luminescent diode is split by a partially reflective mirror into a measuring beam and a reference beam. Reference beam is synchronised with reflected beam resulting in interference which is measured by a photodetector and processed into a signal which can be displayed as an image

4.5.2 Optical Coherence Tomography – Evolution of Identifying Normal Retinal Anatomy

In 1991, Huang *et* al published the first report of the clinical application of OCT to visualise the human retina [278]. A time domain system was employed and was able to resolve to a level of 17um, requiring three minutes to acquire an image of 150 A-scans. Its application was limited to cadaveric tissue and described a hyperreflective zone indicative of a signal from the retinal nerve layer and an outer retinal hyporeflective zone. A second hyper-reflective region distinct from the retina was described and thought to be generated from the retinal pigment epithelium. Two years later a system that could be used *in vivo* was described by Swanson *et al* [279] corroborating the anatomical correlates of the two hyper-reflective zones.

As acquisition speed increased and axial resolution further improved over the following six years, the retinal structure was viewed in greater detail. Scans were able to resolve images of varying levels of reflectivity such that more retinal layers could be distinguished [280-282]. In 2001, ten years after OCT first emerged as a clinical imaging tool, Drexler *et al [283]*reported on ultra-high resolution OCT which represented a major advance in ophthalmic imaging. Where previously OCT systems achieved an axial resolution of between 10-15um, ultra high resolution OCT was capable of an axial resolution of 3um in the human retina. Images generated from this system displayed 3 hyper-reflective bands in the outer retinal region attributed to the photoreceptor outer segments, the RPE and the choriocapillaris.

Four hyper-reflective bands were subsequently reported in 2003 by Zawadzki *et al* [284] in that an additional inner band representative of the external limiting membrane was described. The outermost band was attributed to a signal from Verhoff's membrane, representing the structures where the RPE are joined by tight junctions. The second and third lines were assigned to the modified cilium (the junction between the inner and outer segments of the photoreceptor), and the RPE, respectively.

SD-OCT subsequently gained rapid widespread popularity as a clinical tool for disease diagnosis and management. Its obvious merits as a modality to visualize the human retina almost at a cellular level resulted in a raft of publications describing retinal anatomy in a plethora of disease processes. However, concern existed that inconsistencies both between and within groups raised doubts to the accuracy of the anatomical correlates to which the image bands of varying reflectivity had been attributed [285]. For example, internal to the first outer retinal hyper-reflective band, the outer nuclear and outer plexiform layers are seen on the OCT scan as regions of low reflectivity of markedly different thicknesses. This does not correlate well to histological measurements where the two layers are comparable [286].

Owing to these concerns, and in an attempt to standardise terminology and nomenclature, in 2014 an international panel of imaging and retinal specialists published a report proposing a lexicon for anatomical landmarks of the normal human retina as seen on spectral-domain optical coherence tomography [287]. Figure is reproduced directly from the original publication unaltered and demonstrates the relevant nomenclature.

Figure 4.2: Proposed Lexicon for International Nomenclature of Normal Retinal Image on SD-OCT

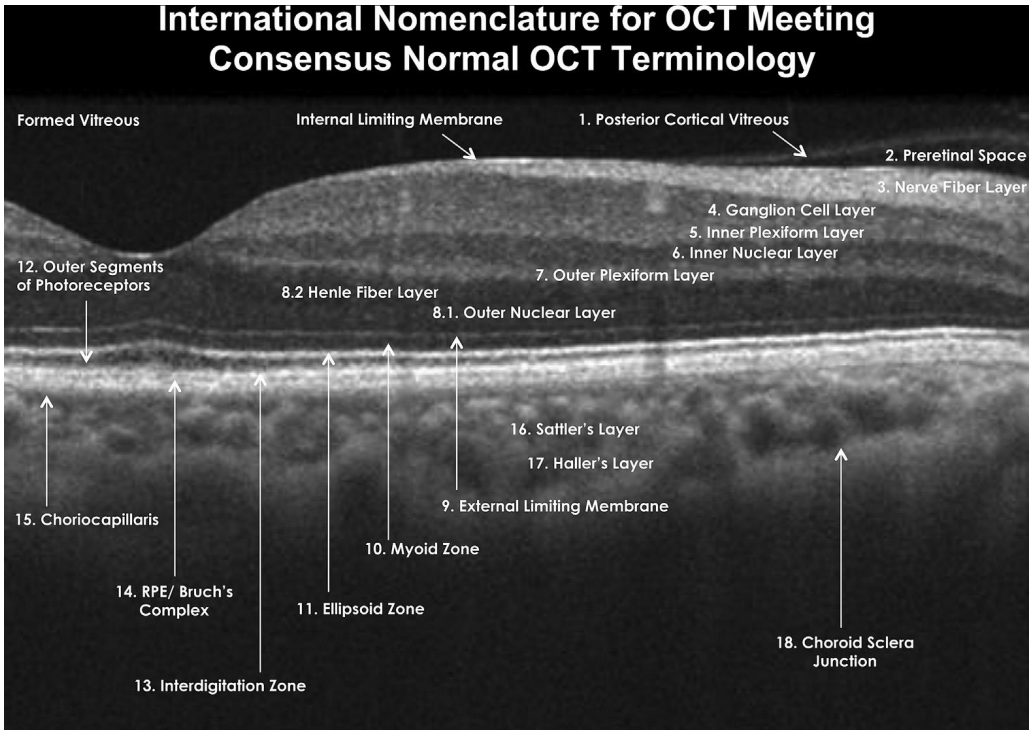


Figure 4.2 Reproduced from Staurenghi et al [287] with permission granted from Elsevier. Note particularly the hyporeflective zone 8.1 (outer nuclear layer), the hyper-reflective band labelled (9) representing the external limiting membrane with band 11 representing the ellipsoid zone (formerly the IS/OS junction)

4.5.3 What happens to photoreceptors after retinal detachment and reattachment?

Experimental models of retinal detachment and subsequent reattachment have shown that the retinal response to neurosensory separation from the RPE is complex. Animal models based on histological findings have shown that the degenerative process occurs progressively inwards from outer retinal structures towards the inner layers. Initially, the apical processes of the RPE undergo morphological changes within a few hours of RRD [288]. Thereafter, the photoreceptor outer segments shorten with subsequent progressive disruption of the inner segments (IS) and IS mitochondria following prolonged duration of detachment.

Apoptosis of photoreceptors has also been described in histological specimens of enucleated eyes following traumatic RDs [289]. Photoreceptor apoptosis has been shown to be induced following retinal detachment in several animal and human studies. Apoptosis has been identified within hours of neurosensory detachment, peaking at day 2 and subsequently dropping after one week. [290-292]

Additionally, complex cellular responses including neuronal synapse remodelling and Müller cell proliferation have also been described.[292, 293] Cellular signalling mechanisms to modify the apoptotic response have been investigated to identify targets for limiting photoreceptor loss and improve visual outcome [290, 294].

Upon subsequent retinal reattachment, the recovery process may therefore require regrowth and reconfiguration of the photoreceptor inner and/or outer segments [295-299], in addition to reorganisation of the cone interdigitation zone CIZ [295, 296].

4.5.4 OCT findings after successful Retinal Detachment Repair

With earlier generation OCT models (Stratus, Carl Zeiss, Meditec, Germany), persistent submacular fluid was reported as a potential cause of incomplete visual recovery following successful RD surgery with vitrectomy and gas tamponade [300, 301] . In 2002, Wolfensberger *et al* [300] observed persistent subretinal fluid (PRF) at the fovea in 11 of 16 eyes at 1 month, eight of which persisted at month 6. In one case they found this still present 12 months postoperatively. Benson *et al* [301] reported on a larger series of 100 eyes and found 15% had evidence of PRF subfoveally at 6 weeks. Eyes with this abnormal finding were followed for up to 18 months with sequential OCT assessments observing a 53% prevalence at month 6. These findings were both replicated [302] and refuted [303] in fovea-off retinal detachments repaired with scleral buckling and the clinical significance of PRF remains uncertain.

In 2008, Smith *et al* [304] reported on the superiority of fourier domain OCT over stratus OCT in this context. Unsurprisingly, due to the superior image resolution, the sensitivity in detecting outer retinal abnormalities post RD repair was significantly higher using the former OCT model. They found photoreceptor disruption in 13 of 17 eyes (76%) imaged with FD -OCT compared to a 12% detection rate (2 of 17 eyes) using the Stratus OCT. Interestingly, the incidence of PRF detection was 12% using both models.

More recently, several groups have focussed on outer retinal abnormalities visualised using SD OCT in eyes postoperatively, and correlated their findings to visual outcomes [305-312]. Both qualitative changes in outer retinal band integrity and quantitative measures of outer retinal band width have been investigated with varying degrees of reproducibility. To date, no such studies have been performed in eyes complicated with PVR.

The Ozurdex in PVR study showed no difference in anatomical outcomes between the two treatment groups (3.2.5.5). There was, however, a difference in rates of cystoid macular oedema at 6 months. CMO was present in 66.2% of eyes in the control arm at this time-point. Benson *et al* [252] observed similar rates of CMO in eyes with poor visual outcome after RD surgery for PVR. A lower incidence (42%) of CMO was noted in eyes which had received the dexamethasone implant in the Ozurdex in PVR study, yet, despite this difference, we did not observe a corresponding better visual outcome in this group at this time-point.

There is, therefore, a clear need to further investigate the cause of limited visual recovery in these eyes which have achieved anatomical success and restoration of 'normal' foveal contour.

4.5.5 OCT Findings in Retinal Detachment

Where previously, descriptions of the RPE and neurosensory response to RRD have relied upon experimental models and/or histological analyses, the advent of OCT has allowed *in vivo* analysis of this process. In 2000, Hagimura *et al* [313] were the first group to report on the pathological changes in the detached neurosensory retina using OCT. Preoperatively, in 25 eyes with fovea-involving RDs, they found a 'normal' foveal structure in 40% (10 of25) with evidence of intraretinal separation in 28% (n=7) and undulation of the separated outer retina in 32% of eyes. More recently, two groups have compared the detached maculae in RRD with acute central serous chorioretinopathy (CSR) using SD-OCT [314, 315]. Both conditions result in neurosensory detachment, but the eyes achieve consistently better vision upon reattachment in latter. Nakanishi *et al [314]* reported similar undulations in the photoreceptor layer in 47% (7 of15) eyes with dropout of the photoreceptor inner and outer segment layers in 6 of 15 (40%) of eyes suffering RRD. These changes were not seen in eyes with CSR and they thus postulated that this finding may be involved in incomplete recovery of vision after RRD repair.

4.5.6 Visual outcomes after successful retinal detachment repair

Despite anatomical success following surgery for fovea-involving rhegmatogenous retinal detachment, suboptimal visual outcomes occur, in eyes which appear normal clinically. In the absence of factors which would obviously explain reduced postoperative visual acuity, e.g. pre-existing macular pathology, amblyopia, postoperative media opacity, recent studies have correlated outer retinal abnormalities as seen on SD –OCT with poor visual outcome [305, 312]. These findings may offer insight into therapeutic targets for neuroprotective or regenerative strategies.

4.5.7 Hypothesis

In eyes that have undergone RD surgery with PVR, there are changes in the outer retina which can be visualised using Spectral Domain Optical Coherence Tomography (SD-OCT) and can be correlated to visual recovery at 6 months post repair.

4.5.8 Methods/Design

This was an observational, non-comparative, cross sectional study of eyes with a normal foveal contour as imaged using SD-OCT at 6 months post study vitrectomy in the Ozurdex in PVR Study.

Of the 140 patients who underwent vitrectomy surgery 138 patients were managed with silicone oil. In six patients, an OCT scan was not performed either due to non-attendance (lost to follow up) or fundus obscuring media opacity. A further 7 eyes had neurosensory retinal detachments present. Seventy four eyes with CMO and 5 eyes with a history or presence of a full thickness macular hole were also excluded. Retained Silicone oil and marked myopic foveal atrophy excluded a further four eyes. Of the remaining 42 eyes, a further 15 eyes were excluded due to co-existing ocular

pathology limiting visual outcome e.g. amblyopia, macular degeneration, visually significant media opacity. Four eyes were fovea-sparing at the time of their study vitrectomy and were also excluded.

Therefore, a total of 22 eyes with fovea-involving RDs complicated with PVR and anatomical success at 6 months were included in the study. This group of 22 eyes comprised twice as many adjunct patients (n=15) as control patients (n=7).

4.5.8.1 *OCT scanning protocol*

The OCT scanning protocol sequence has been outlined in the study protocol section 3.2.4.5.5.

4.5.8.2 *Interpretation of OCT Scan*

Scans were viewed on an HP ProDisplay P201 monitor (20 inches) with a resolution of 1600 x 900. The B scan displaying the deepest foveal excavation was chosen for analysis. The International Nomenclature for OCT (IN-OCT) Consensus was used as the reference for identification of anatomical layers and zones as proposed by Staurenghi *et al* [287]. For the purposes of this study the following binary variables were identified as either present or absent, a) discontinuity of zone 9 (the External Limiting Membrane (ELM)), b) discontinuity of zone 11 (the ellipsoid zone of the photoreceptors), c) the presence of discrete hyper-reflectancies in zone 8 (the outer nuclear layer) and d) abnormal hypo-reflectancies in zone 12 (the outer segments of the photoreceptors). Four continuous variables were investigated as follows: a) foveal thickness, b) macular volume, c) outer nuclear layer thickness (zone 8) and d) photoreceptor outer segment thickness (zone 12). Both foveal thickness and macular volume measurements were obtained using automated segmentation algorithms incorporated into the Heidelberg software. ONL and PROS thickness required manual segmentation using the measuring calliper setting.

Figure 4.3: External Limiting Membrane Disruption and Photoreceptor Outer Segment Abnormalities on SD-OCT

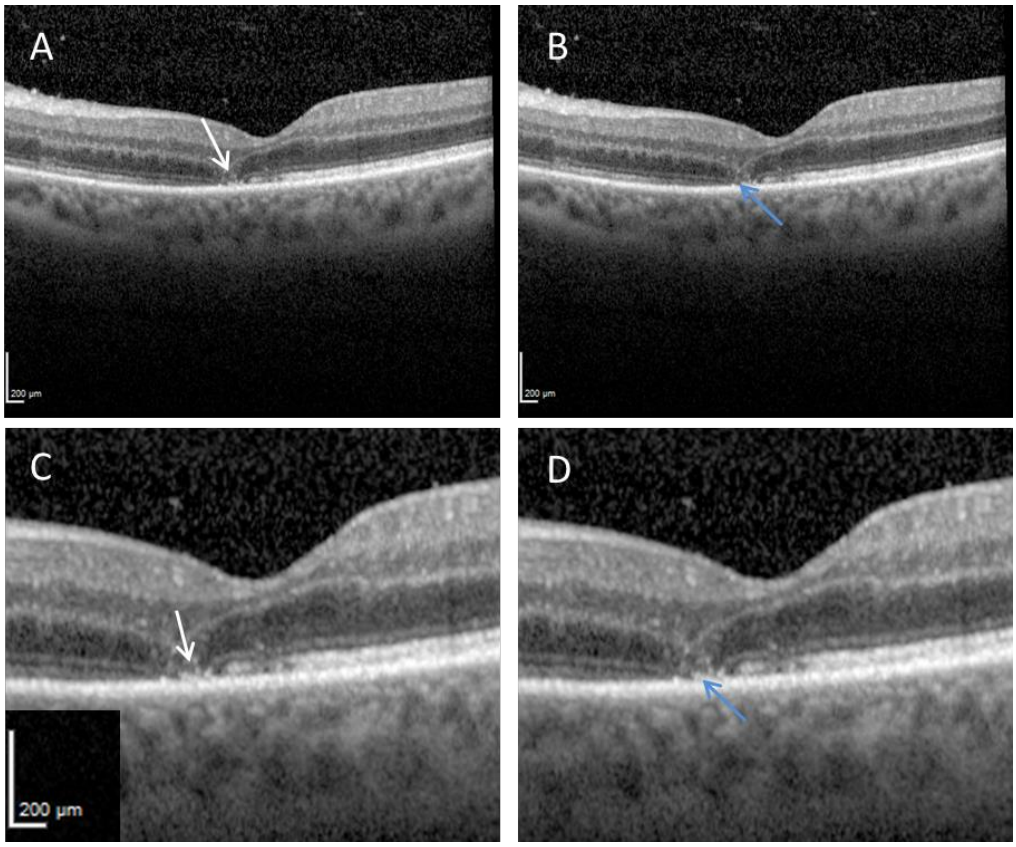


Figure 4.3 (A) and (B) Example of discontinuity/disruption of the external limiting membrane (ELM) (white arrow) and photoreceptor outer segment abnormal reflectancies (blue arrow). (C) and (D) show the abnormal reflections under higher magnification (scale bar 200µm)

Figure 4.4: Outer Nuclear Layer and Ellipsoid Layer Abnormalities on SD-OCT

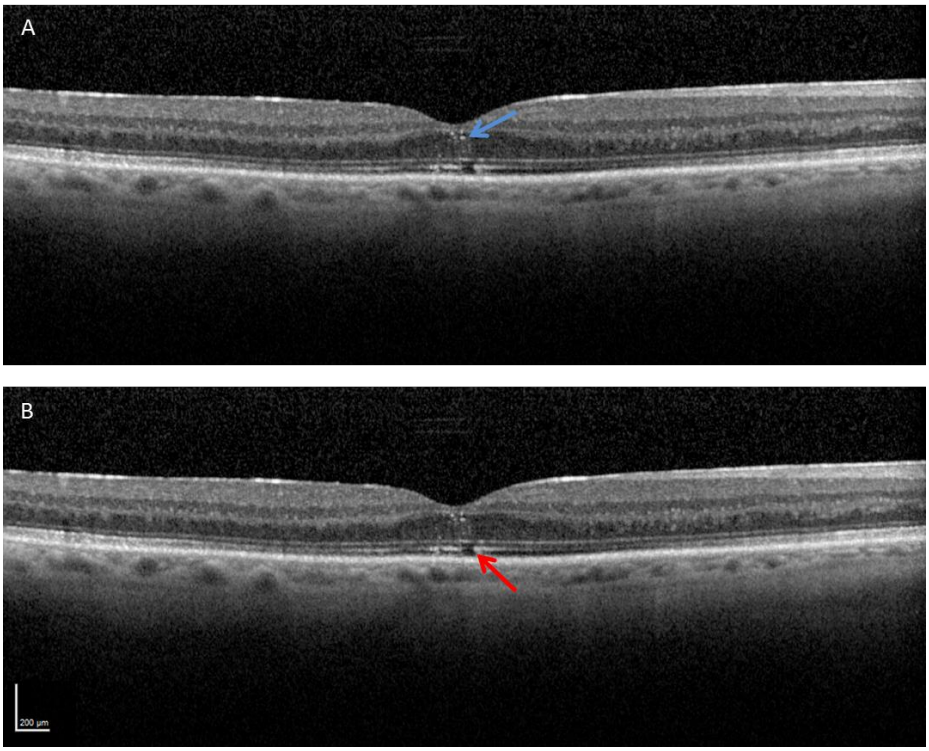
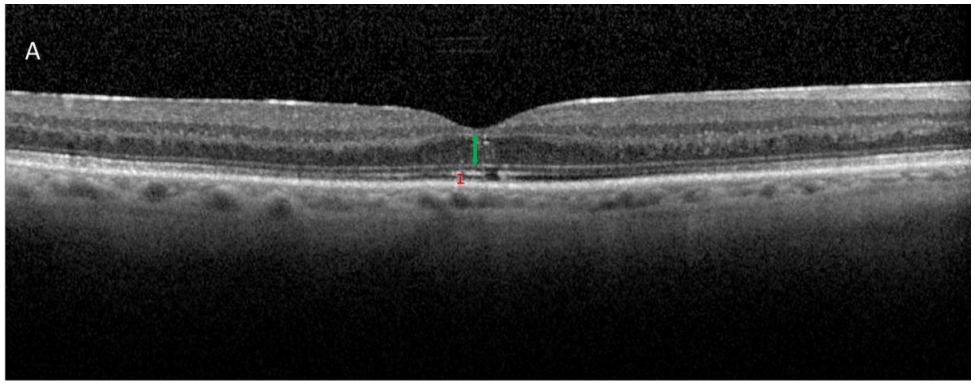
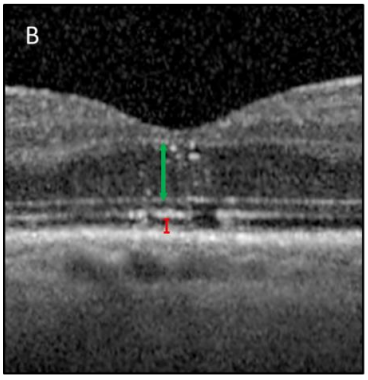


Figure 4.4 (A) Blue arrow indicates discrete hyper-reflective lesions in the outer nuclear layer (ONL) (B) red arrow indicates discontinuity of the ellipsoid layer

Figure 4.5: Technique for Manual Segmentation of Outer Retinal Layers.





(A) and (B) demonstrate technique for manual segmentation with the caliper function incorporated into the Heidelberg software. Double headed green arrows segment the outer nuclear layer (ONL) from the ELM to the outermost border of the outer plexiform layer , the red bars segment the photoreceptor receptor outer segment (PROS) layer from the outer border of the ellipsoid layer to inner border of the RPE/Bruch's complex . The borders of the ONL are better defined than the less distinct borders of the PROS.

4.5.9 Statistical Analysis

IBM SPSS version 22 was used for the analysis. Univariate linear regression analysis was performed for all of the aforementioned independent variables using ETDRS visual acuity at 6 months post study vitrectomy as the continuous dependent variable. Additionally, preoperative visual acuity and baseline grade of PVR (highest recorded grade intraoperatively at the time of study vitrectomy) were also included as independent variables. Forward stepwise multiple regression analysis was then performed for all continuous and binary independent variables significant where $p < 0.05$. The final model fitted contained variables significant where $p< 0.05$. Analysis of variance (ANOVA) was used to compare mean postoperative visual acuities by variable.

4.5.10 Results

Twenty two eyes of 22 patients were analysed. There were an equal number of male and female patients with a right eye preponderance (59.1%). Median baseline visual acuity was zero ETDRS letters. The median best corrected final visual acuity was 63 ETDRS letters and median grade of PVR at presentation was grade 3. Discrete hyper-reflectant changes in ONL were found in 11 eyes (50.0%). Discontinuity of the ELM was observed in 6 eyes (27.3%). The ellipsoid layer was disrupted in 15 of the 22 eyes (68.2%) of eyes. Detecting abnormal hyporeflectancy in the photoreceptor outer segment (PROS) layer was challenging and deemed unreliable in 8 eyes. It was thought to be present in 12 of the remaining 14 eyes (85.7%). The mean PROS thickness was 28.5 µm. Mean central foveal thickness (FT) was 260 µm (SD 38.5) and mean macular volume (MV) was $8.33mm^3$(SD 1.13). The mean thickness of the outer nuclear layer (ONL) was 96.2 µm (SD 26.7).

Table 4.1: Patient Demographics and Postoperative SD-OCT findings

No	Sex	Eye	Final VA	Base VA	PVR Grade	ONL abnorm	ELM (disrupt)	Ellipsoid (disrupt)	PROS changes	FT µm	MV mm^3	ONL µm	PROS µm
1	F	OD	44	0	4	+	+	+	+	220	7.93	66	26
2	F	OS	51	59	4	-	-	-	-	254	9.06	87	27
3	F	OS	41	0	2	+	+	+	NP	194	6.98	58	12
4	M	OD	71	15	2	+	-	+	+	312	5.92	117	29
5	M	OS	70	15	3	-	-	+	NP	303	9.49	125	23
6	M	OD	36	0	4	+	+	+	NP	220	8.6	64	23
7	F	OS	77	0	3	-	-	-	+	259	8.84	118	32
8	M	OS	35	7	3	+	-	-	NP	232	8.5	66	28
9	F	OD	78	44	4	-	-	-	+	215	7.69	82	28
10	F	OS	63	53	3	-	-	+	NP	314	8.9	119	NP
11	M	OD	63	0	4	-	-	-	+	297	8.34	153	29
12	F	OD	65	39	4	+	-	+	NP	222	7.52	87	23
13	M	OD	63	21	3	+	-	+	+	236	8.74	76	32
14	M	OD	74	0	2	-	-	-	-	293	8.4	128	39
15	M	OD	70	40	2	-	-	+	+	243	5.09	79	33
16	F	OD	74	0	4	+	-	+	+	231	8.65	102	23
17	M	OS	36	0	4	+	+	+	NP	284	8.8	77	30
18	M	OD	58	0	6	+	+	+	+	316	9.99	90	21
19	F	OS	74	11	6	-	-	-	+	310	9.58	136	34
20	F	OD	65	0	5	-	-	+	NP	291	8.6	116	37
21	F	OS	60	0	3	+	-	+	+	237	8.32	97	46
22	M	OD	51	53	2	-	+	+	+	239	9.3	74	25

VA =Visual Acuity in ETDRS letters, Base = baseline, PVR = proliferative vitreoretinopathy, ONL abnorm = Outer nuclear layer hyperreflectancies, PROS changes = photoreceptor outer segment hyporeflectancy, FT = foveal thickness, MV = macular volume, ONL = outer nuclear layer, PROS = photoreceptor outer segments

Table 4.2: Linear Regression Analysis

Independent Variable	R squared	coefficient	Standard error	T score	P value	95 % Confidence Interval
Baseline VA	0.026	0.107	0.147	0.730	0.474	-0.20 to 0.41
Baseline PVR Grade	0.000	0.222	2.655	0.083	0.935	-5.32 to 5.76
ONL changes	0.256	-13.909	5.304	-2.622	0.016	-24.98 to -2.85
ELM Disruption	0.484	-21.479	4.959	-4.332	0.000	-31.82 to -11.14
Ellipsoid Disruption	0.053	-6.771	6.425	-1.054	0.304	-20.17 to 6.63
PROS change	0.178	11.650	5.598	2.081	0.05	-0.03 to 23.33
Foveal Thickness	0.115	0.125	0.082	1.529	0.144	-0.05 to 0.30
Macular Volume	0.014	-1.432	2.810	-0.509	0.617	-7.34 to 4.47
ONL thickness	0.451	0.356	0.088	4.051	0.001	0.17 to 0.54
PROS Thickness	0.128	0.716	0.428	1.670	0.111	-0.18 to 1.61

Table 4.3: Multiple Variable Regression Model

Independent Variable	R squared	coefficient	Standard error	t	P value	95 % Confidence Interval
ELM Disruption	0.479	-17.636	2.460	-3.32	0.006	-29.21 to -6.06

The mean visual acuity in eyes with an intact ELM at six months was 65.8 +/- 2.7 ETDRS letters compared to 44.3 +/- 3.6 ETDRS letters in eyes with evidence of ELM disruption ($p<0.001$). (Figure 4.6)

Figure 4.6: Blox plot of Visual Acuity Depending on Integrity of ELM

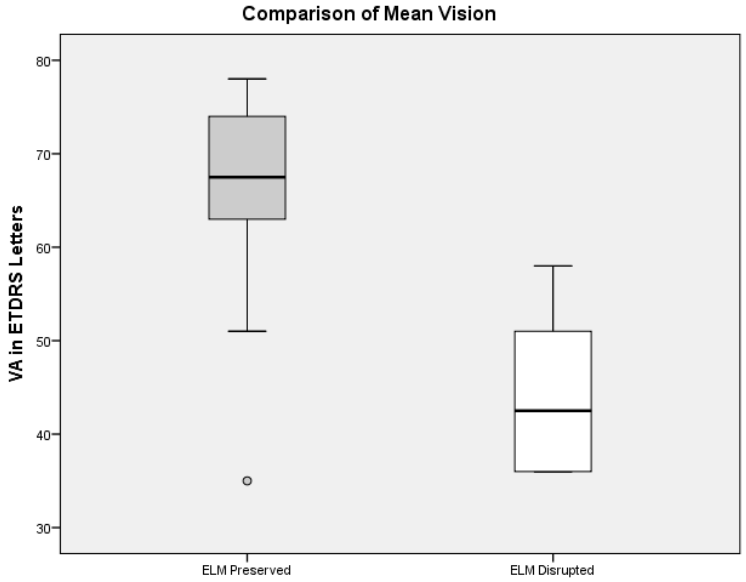


Figure 4.6 demonstrates that in eyes with successful PVR retinal detachment repair, achieve a better visual outcome (mean 65.8 , standard deviation 2.5) where the external limiting membrane is preserved compared to eyes where there is evidence of disruption of this band (44.3 letters +/- 3.6). (central bar = median, box = interquartile range, whiskers = range)

4.5.11 Discussion

Despite successful anatomical reapposition of the neurosensory retina to the RPE, the visual outcome in eyes which have suffered foveal detachments remains unpredictable and sometimes unsatisfactory. Numerous studies have correlated several factors predictive of final visual acuity including preoperative VA [316], height [316, 317] and duration of detachment [316, 318]. Improvements in imaging modalities have facilitated the detailed visualisation of retinal microstructure such that the resolution achieved nears histological clarity. Several authors have used OCT to correlate postoperative retinal appearance with visual outcome.

In our series, we did not find persistent subfoveal fluid present at 6 months in any of the eyes which were imaged. This may indicate that eyes without PVR reattach in a different manner to eyes where PVR is present. This pertinent negative finding, further questions the clinical significance of PRF as an indicator of limited visual recovery. Its previously observed presence may, in fact, have simply co-existed with more subtle outer retinal changes which were then yet to be defined due to limitations in imaging resolution.

In 2009, Wakabayashi *et al [311]* reported the significance of the combined discontinuity of the ELM band and the ellipsoid layer (then referred to as the inner segment (IS) and outer segment (OS) junction), in relation to postoperative visual outcome. Their cohort of 53 eyes consisted of 15 fovea- sparing and 38 fovea-involving RDs. Two thirds underwent pars plana vitrectomy with the remaining third managed successfully with scleral buckle surgery. Photoreceptor layer abnormalities were only observed in the fovea-involving sub group. No photoreceptor layer abnormality was observed in 15 of the 38 eyes (39.5%). Ellipsoid layer disruption with an intact ELM was noted in 14 of the 38 eyes (36.8%). Disruption of the ELM was always noted in conjunction with ellipsoid layer discontinuity, and was present in 9 eyes (23.7%). The mean best corrected visual acuity was significantly lower ($p<0.001$) in the group with ELM disruption 0.6LogMAR +/- 0.32 compared to eyes with an isolated ellipsoid layer

abnormality (0.34+/- 0.15) and those without any detectable photoreceptor abnormality (LogMAR -0.03+/-0.10). This mirrors our findings in eyes with PVR where the ELM integrity was the only variable significant on the multiple variable regression model. We also found a statistically significant difference ($p<0.001$) on comparison of mean post op VA in eyes with and without ELM disruption (44.3 +/- 3.6 ETDRS letters vs 65.8+/- 2.7 ETDRS letters respectively). However, it must be acknowledged that as the inclusion required a preserved foveal contour, we may have selected scans of eyes with PVR representative of less severe foveal pathology. Extrapolating this finding more broadly may therefore be limited.

Wakabayashi *et al* [311] found recovery of the ellipsoid layer in approximately two thirds of eyes with preserved ELM integrity but no evidence of reorganisation of either the ellipsoid layer or ELM in eyes where the latter had been affected. Their findings supported the histological evidence that the process of neurosensory retinal degeneration occurs progressively inwards. They also concluded that involvement of the external limiting membrane may be indicative of irreversible damage to the photoreceptor cell bodies and Muller cell cone and that it may be predictive of restoration of photoreceptor microstructure after RD repair.

In 2010, three groups published their findings [307, 309, 319], two of which concentrated only on the integrity of the IS/OS junction (ellipsoid layer) [309, 319]. Both Sheth *et al* [309] and Shimoda *et al* [319] correlated discontinuity in the ellipsoid layer with limited postoperative visual recovery, but the absence of comment on the integrity of the ELM makes it difficult to draw direct comparisons to our study. In addition to SD-OCT, Lai *et al* [307] introduced microperimetry and fundus autofluorescence findings in their correlation with visual outcome. Again drawing direct comparison with our findings is difficult due to differing methodology, particularly as our dependant variable remained continuous where Lai *et al* dichotomised visual outcome into optimal, > 0.52LogMAR (6/18 Snellen equivalent) and suboptimal ≤ 0.52LogMAR. Individual abnormalities in any of the outer retinal

bands (ELM, ellipsoid and cone interdigitation zone) were significantly associated with suboptimal postoperative visual outcome. They also noted that the areas of decreased microperimetric sensitivity corresponded well with both the ultrastructural OCT changes and abnormal FAF.

In 2012, in addition to the aforementioned binary qualitative outer retinal OCT findings, three groups introduced quantitative measures [305, 308, 312] as potential correlates to visual recovery. Kim *et al* [312] found the ONL thickness in reattached retina to be significantly thinner than that in the unaffected retina of the same eye, when measured equidistant from the foveal centre. The significance of the ONL thickness in relation to visual outcome was reported by Gharbiya *et al* [308] in their study of 23 fovea-involving primary RDs. They report a mean post ONL thickness of 105µm +/- 25.2, which is similar to our findings (96.2 µm +/- 26.7). Although ONL was significant in univariate regression, it subsequently lost significance in our multiple regression model. Delolme *et al* [305] did not find a significant correlation of ONL thickness and final VA. However, unlike our findings, they reported the significance of the PROS thickness. This will be discussed further subsequently when commenting on limitations of our study. Interestingly, Delolme *et al* found no difference in visual acuity between those with ellipsoid layer disruption and preserved ELM and those with disrupted ellipsoid layer with ELM disruption

The anatomical significance of the ELM and its potential significance to visual recovery will be further expanded in section 4.6.4

4.5.11.1.1 Limitations

Although the numbers included in this study are small they are comparable to published studies of similar design. The sample is a heterogenous group, particularly in terms of number of previous procedures and number of times the fovea was detached but is reflective of an unselected group of eyes with attached retinae and preserved contour at 6 months post intervention. Furthermore, in this study, all comparisons are made to published findings on eyes without PVR.

Despite this, the inclusion criteria were still relatively restrictive so as to exclude alternative causes of visual loss. Furthermore it was necessary to eliminate variables such as CMO and ERM which could have contributed to changes in retinal layer thickness and are an obvious cause of visual impairment. The process of scan selection could therefore be questioned, as it is likely to favour those eyes with better vision. However, it could be argued that focussing on this group of eyes may be of specific importance, especially if by improving the modifiable secondary causes of visual loss (e.g CMO) we are able to maximise the number of eyes with 'normal' foveal contour.

The cross sectional study design does not give an indication of change over time and this could be further analysed in future studies.

The manual segmentation of photoreceptor outer segment (PROS) layer was challenging. Although the inner border (the outer limit of the ellipsoid layer) was usually well defined, the outer border was less easily visualised (Figure 4.5). Further potential inaccuracies may occur when there is ellipsoid layer discontinuity and the reliability of this measurement could be questioned.

Additionally, the manual segmentation of ONL and PROS and the grading of qualitative measures are subjective. Repeat assessment of these images both by myself and an independent observer, may help to determine the test-retest variability and provide a measure of inter and intra observer agreement. This may serve to validate my findings.

Finally, the reliability of hyperreflective outer band continuity as an indicator of tissue damage has been questioned. The absence or fragmentation of the ELM, ellipsoid zone and the cone interdigitation zone have been reported as artefact in normal eyes [320]. The ability to visualise a band as a discrete structure is a function of multiple factors. Amongst others, this includes the thickness of the flanking layers which border the band [319]. Fortunately, these quantitative values were measured in this study, but the reliability and reproducibility of the PROS measurements could be questioned.

4.6 Primary PVR and Primary Surgical Success

Where the work conducted so far on PVR eyes with normal foveal contour post successful retinal detachment repair at 6 months has provided interesting results, it is limited in both the heterogeneity of the sample and the cross sectional design of the study. Information regarding recovery of band discontinuity and change in retinal layer thickness may further facilitate our understanding of the visual recovery process in these complex eyes.

Therefore, in order to explore this recovery process over time in a homogenous group of eyes with PVR, a longitudinal study design is required. Eyes with primary PVR are those where no previous surgery has been performed. Furthermore, focussing on eyes where primary anatomical success has been achieved may further improve group homogeneity.

4.6.1 Aim and hypothesis

To correlate postoperative visual acuity with postoperative SD-OCT findings in eyes with primary PVR fovea-involving RDs achieving primary anatomical success.

To explore whether outer retinal abnormalities as visualised using SD-OCT change over time and provisionally correlate this to visual outcome

4.6.2 Methods

Of the 140 eyes in Ozurdex in PVR Study, 41 eyes presented with primary PVR. Twenty one eyes achieved primary anatomical success, two of which were fovea sparing and two treated with gas. Seven eyes with ocular co-morbidity limiting visual outcome were excluded (two eyes were amblyopic, two had macular degeneration, two had previous macular surgery and one patient there was a visually significant corneal scar). Longitudinal analysis of the remaining 10 eyes was performed and is herein described.

The OCT scanning protocol and scan interpretation has previously been outlined in section 4.5.8.1.

As the sample size is small, the results will be presented as a descriptive series from baseline to 12 months post retinal detachment repair. Statistical comparisons have not been made. This sample of ten patients with primary PVR consisted of five patients who had received the adjunct and five control patients. This equal distribution occurred by chance and did not form the basis of the sample selection.

4.6.3 Results

Table 4.4: Summary of Ocular and Non-ocular Baseline Characteristics of the Patients

No	Sex	Eye	AGE (Yrs)	Ethnicity	Base VA ETDRS Letters	Spher Equiv.	Lens Status	No. of Breaks	RD Ext. (hrs)	RD dur. (days)	PVR Grade
1	M	OS	63	Black	21	-0.25	PCIOL	2	8	365	3
2	F	OS	73	Caucasian	3	-0.75	PCIOL	4	12	18	2
3	M	OD	66	Caucasian	0	+ 0.5	CLEAR	2	10	10	3
4	M	OD	65	Caucasian	15	NP	PCIOL	2	7	18	4
5	M	OD	64	Caucasian	40	-3.75	NS+	1	3	NP	1
6	F	OD	73	Caucasian	65	0.0	NS+	1	4	21	2
7	F	OD	61	Caucasian	0	+1.25	PCIOL	1	9	5	4
8	M	OS	53	Caucasian	42	+0.25	CLEAR	1	8	NP	4
9	F	OS	77	Caucasian	0	-0.68	PCIOL	2	12	28	2
10	F	OS	85	Caucasian	0	NP	PCIOL	1	8	90	3

Spher Equiv. = spherical equivalent, No.of Breaks= number of breaks, RD ext (hrs) = extent of retinal detachment on clock hours, RD duration = duration of RD, PVR Grade = maximum recorded grade of PVR C (either posterior or anterior), NP = not possible/not recorded, PCIOL = posterior chamber intraocular lens, NS = nuclear sclerosis

4.6.3.1 *Presenting features*

The baseline characteristics are displayed in Table 4.4. demonstrating an equal sex distribution and a mean age of 68.4 years. Presenting visual acuity was poor in the majority of the ten cases, with 4 out of 10 patients unable to see any ETDRS letters. One patient (case 6) presented with a fovea bisecting retinal detachment and hence had a better presenting visual acuity. Six patients were pseudophakic; two had clear lenses and two early nuclear sclerotic cataracts. The median grade of PVR was Grade C3. The reported duration of RD ranged considerably, from 5 days to one year. In two patients the duration of RD was not recorded.

4.6.3.2 *Visual Outcome*

Figure 4. displays the trend of visual recovery for each patient over the 12 month follow up period. The final visual outcome was good in most patients, with an observed median final vision of 72 ETDRS letters at 12 months (range 44 to 80 letters). In order to better graphically visualise the recovery of vision over time Figure 4. has been further separated into two sub figures, both containing 5 patients. From these images it is clearer to see that in the majority of cases the greatest visual improvement appears to occur between baseline and Day 10 post repair. Thereafter, there is further improvement up to month 3, with a plateau after month 6.

Figure 4.7: Line Graph of Visual Acuity Progression in Eyes with Primary PVR

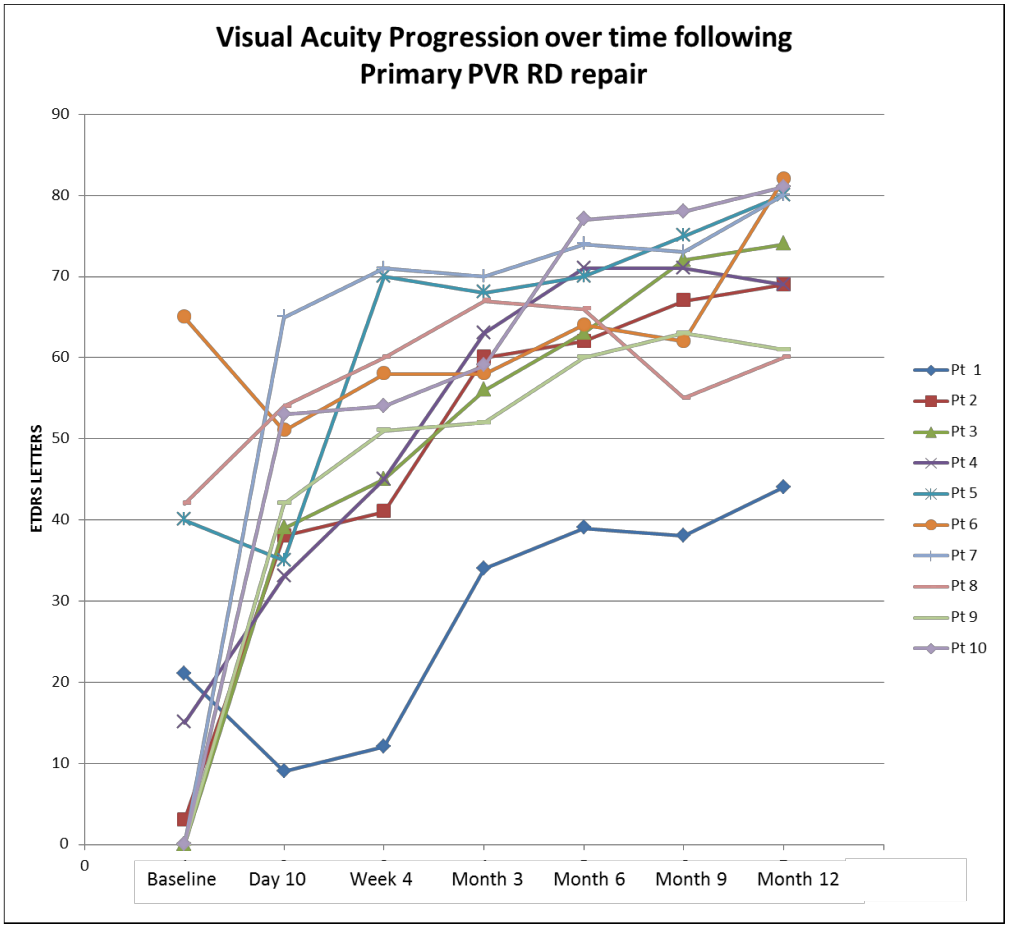
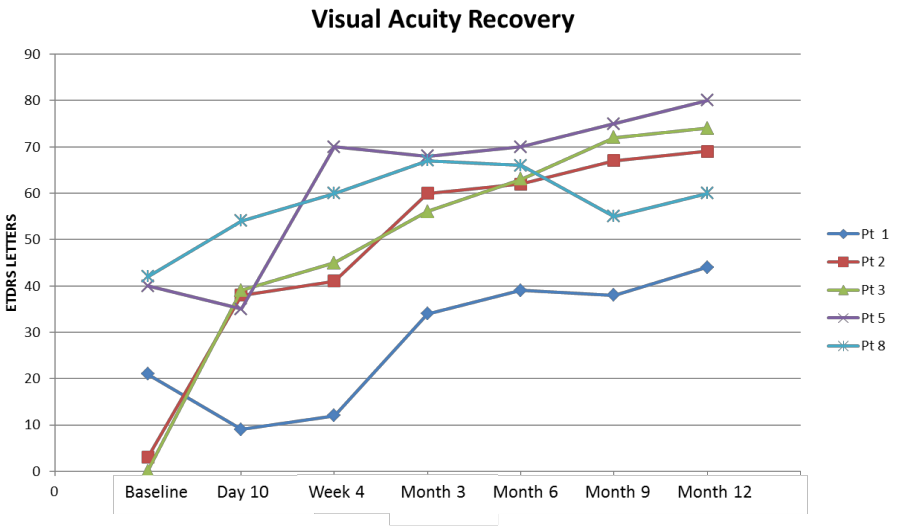


Figure 4.8: Two Line Graphs of Visual Recovery in Eyes with Primary PVR



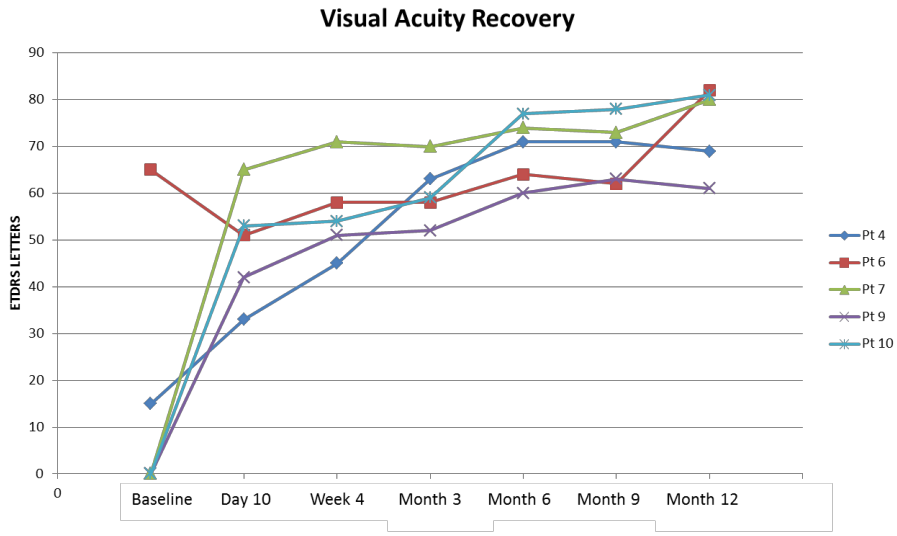


Figure 4.8 Two line graphs arbitrarily divided into two groups for clarity of display. In general, the greatest improvement occurs initially from baseline to Day 10, and up to month 6. Thereafter, the visual recovery appears relatively static between months 6 and 12.

4.6.3.3 *Description of Cases*

Selected cases will be outlined sequentially, and where common features present, these will be elaborated further in the discussion.

4.6.3.3.1 Patient 1

Figure 4.9: SD OCT Sequence of Patient 1

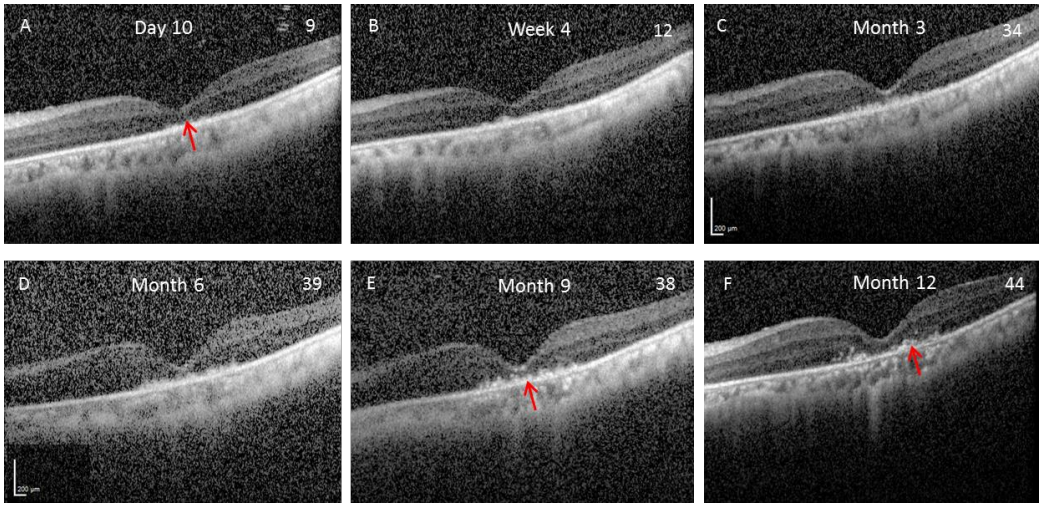


Figure 4.9 . A 63 year old African male underwent a primary retinal detachment (RD) repair with silicone oil. Duration of the RD was estimated as one year. (A) to (F) demonstrate the sequential SD-OCT scans from day 10 post repair with oil to month 12 (Scale Bar 200µm, numbers in upper right corner of each box of composite display the visual acuity in ETDRS letters). (A) Day 10 post RD repair with oil shows marked disorganisation of the outer retina. Discrete hyperreflectancies (red arrow) are seen inner to the RPE layer but with no discernible resolution of the outer retinal bands representative of ELM and ellipsoid layers. VA is 9 ETDRS letters. (B) and (C) Week 4 and Month 3 post-op showing mild recovery of vision but persistent outer retinal disorganisation (D) to (F) the oil has been removed but the vision remains poor; scans suggest possible partial reorganisation of the ONL but no recovery of the ELM or ellipsoid zone.

4.6.3.3.2 Patient 2

Figure 4.10: SD-OCT sequence of Patient 2 (a)

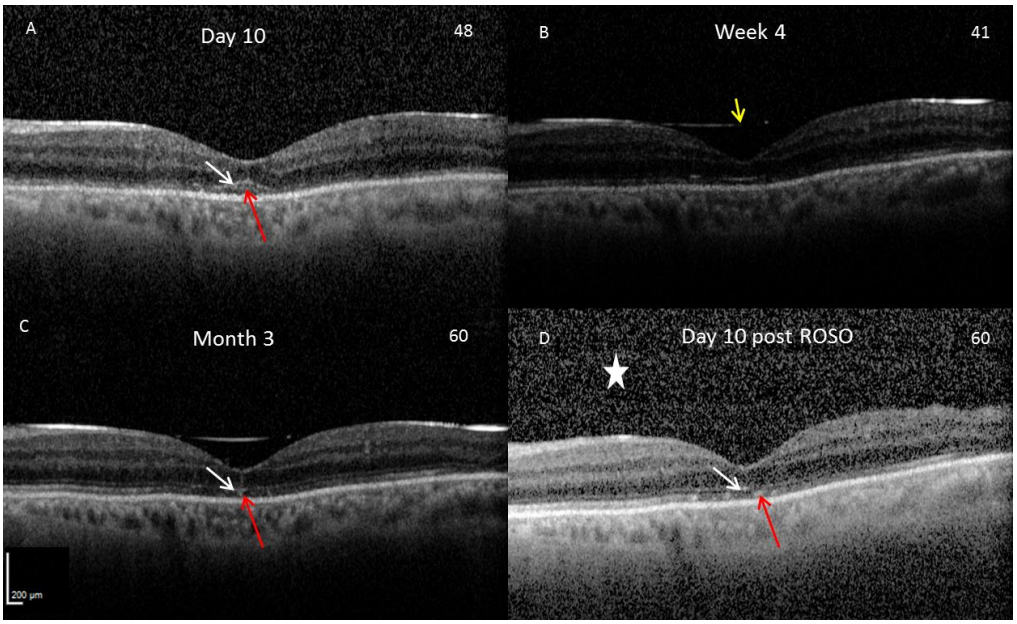


Figure 4.10 76 year old pseudophakic Caucasian female presented with a primary PVR RD of 18 days duration. She underwent a repair with oil. (A) to (D) demonstrate the sequential SD-OCT scans from day 10 post repair with oil to day 10 post oil removal at month 4 (Scale Bar 200µm, numbers in upper right corner of each box of composite display the visual acuity in ETDRS letters). (A) At day 10 post RD repair, the retina is attached with disorganisation of the outer retinal hyper-reflective bands, perhaps with evidence a residual subfoveal bleb of subretinal fluid. VA is 48 ETDRS letters. (B) By week 4, a hyper-reflective outer retinal band (white arrow) most likely representative of the ELM is visible but incomplete; VA remains poor at 41 ETDRS letters (yellow arrow shows hyper-reflective signal from posterior meniscus of oil bubble). (C) By month 3 the VA is now 60 ETDRS letters with a distinct ELM (white arrow) but disrupted ellipsoid layer (red arrow). (D) Ten days post oil removal the changes observed in figure C are clearer and the vision is unchanged. Note also the increased number and intensity of pixels in the vitreous (white star)

Figure 4.11: SD-OCT sequence of Patient 2 (b)

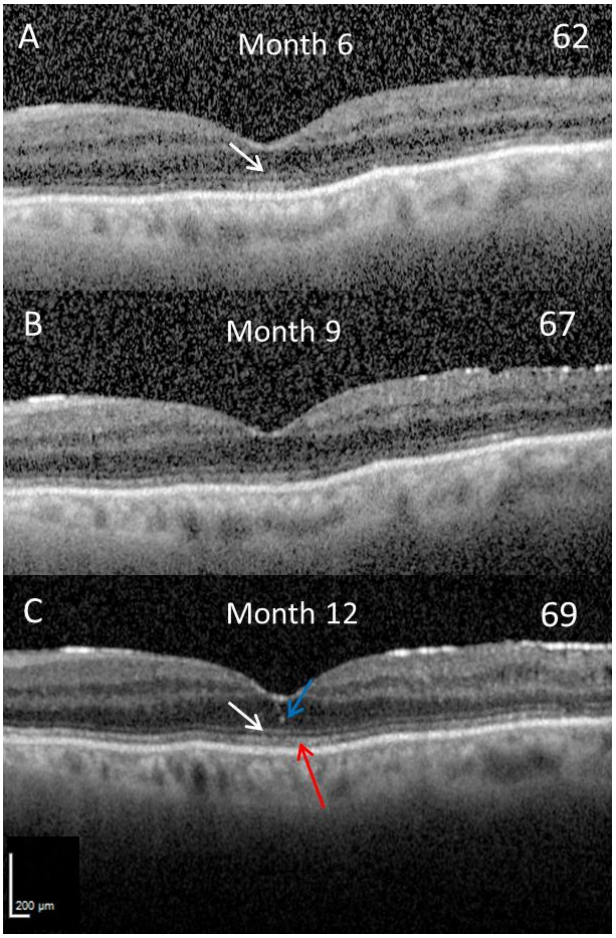


Figure 4.11 Figure (F) to (G) show sequential SD-OCT scans at months 6, 9 and 12 of patient 2(scale bar 200µm). Note how the visual acuity remains static as the hyper-reflective band representing the ELM remains intact whilst the ellipsoid layer continuity improves (C) there are discrete hyper-reflectant lesions in the outer nuclear layer (blue arrow), the ELM is continuous and there may be subtle disruptions in the ellipsoid layer. VA is 69 letters at month 12

4.6.3.3.3 Patient 4

Figure 4.12: SD-OCT Sequence of Patient 4

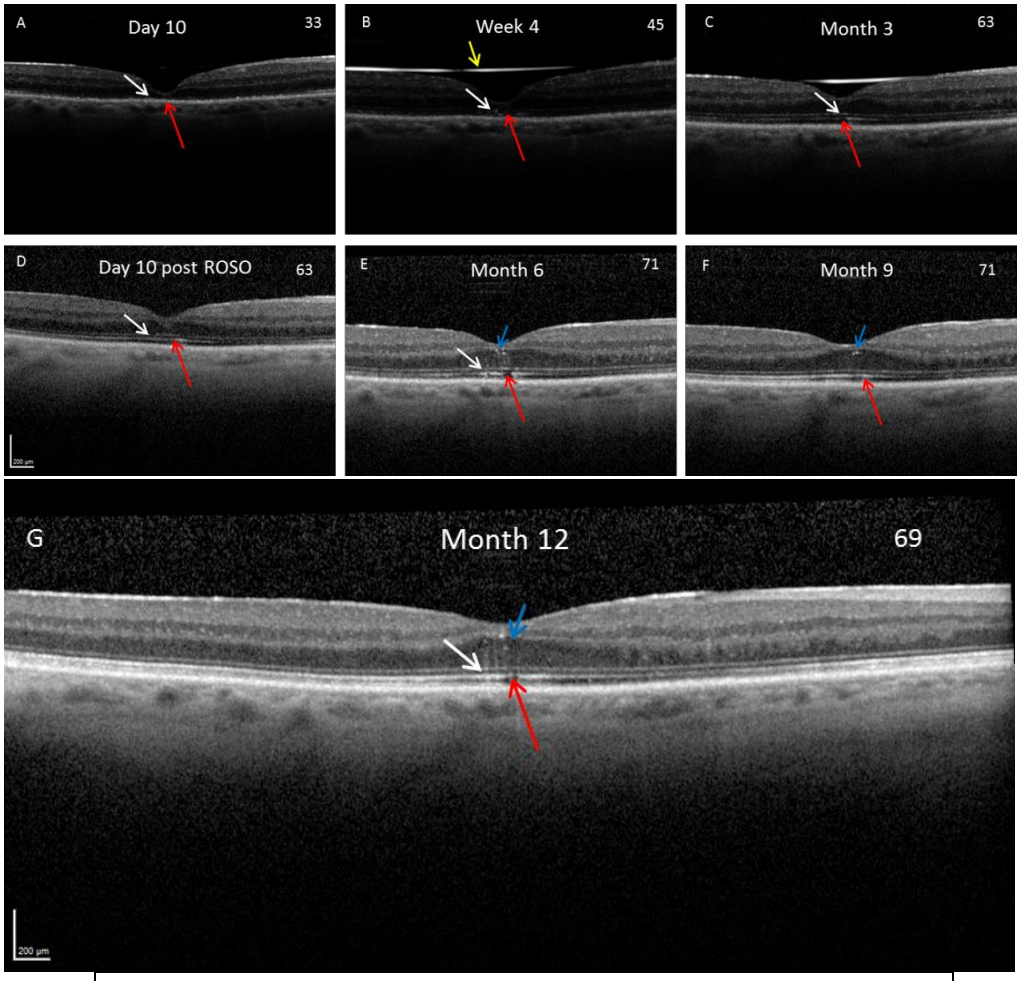


Figure 4.12 A 65 year old pseudophakic Caucasian male presented with a primary PVR RD (Grade CP4) of 18 days duration. (A) to (G) demonstrate the sequential SD-OCT scans from day 10 post repair with oil to month 12 (Scale Bar 200µm, numbers in upper right corner of each box of composite display the visual acuity in ETDRS letters). (A) and (B) demonstrate poor resolution of the ELM (white arrow) and ellipsoids layer (red arrow) with a barely discernible outer nuclear layer. ETDRS VA remains poor (yellow arrow indicates hyper-reflection from posterior oil meniscus). (C) By month 3, VA has improved to 63 letters as the ELM continuity is restored (white arrow) and ellipsoid layer is visible but discontinuous (D) the ellipsoid layer (red arrow) appears intact day 10 after oil removal but shows signs of discontinuity at month 6 in figure E . (E) to (G) Months 6, 9 and 12 the VA remains stable with a continuous ELM (white arrows), ellipsoid layer with disruptions (red arrows) and hyper-reflective lesions in the ONL

4.6.3.3.4 Patient 5

Figure 4.13: SD-OCT Sequence of Patient 5

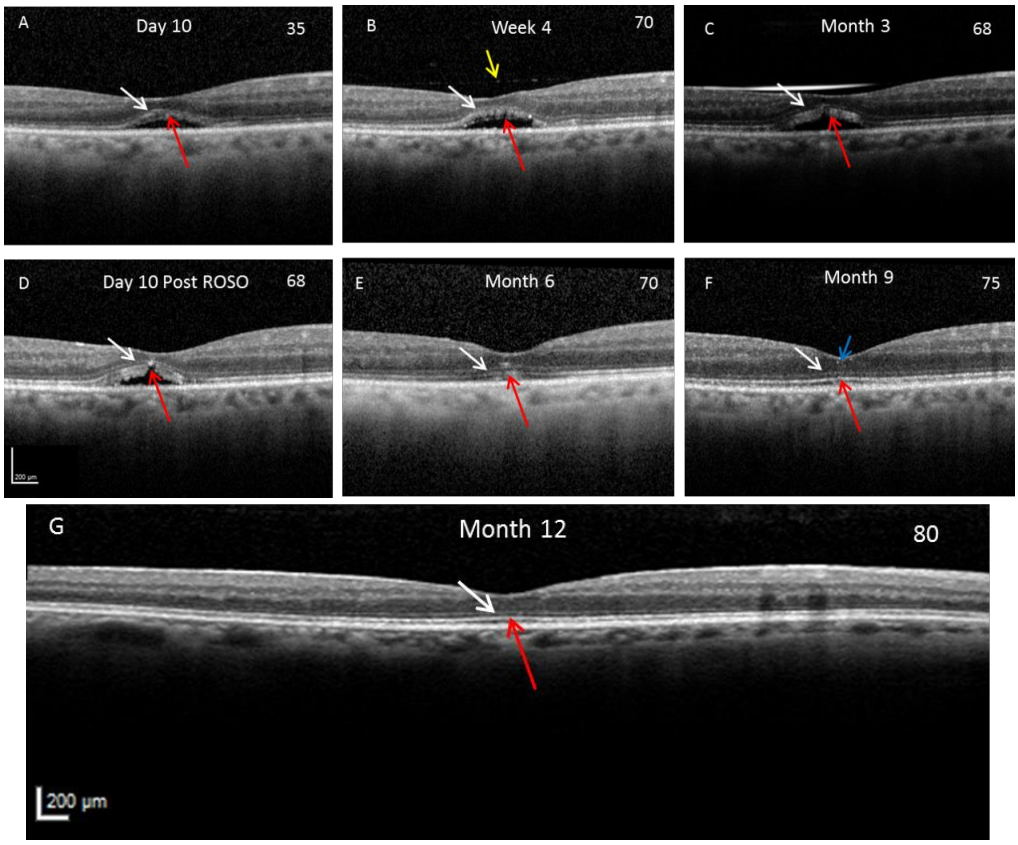


Figure 4.13 A 63 Caucasian male with early nuclear sclerotic cataract underwent a primary PVR RD repair. (A) to (G) demonstrate the sequential SD-OCT scans from day 10 post repair with oil to month 12 (Scale Bar 200µm, numbers in upper right corner of each box of composite display the visual acuity in ETDRS letters). (A) Day 10 post RD repair there is disorganisation of the outer retinal layers with no ELM band continuity, a disrupted ellipsoid zone and a bleb of subfoveal fluid (black region below red arrow); corresponding VA is poor at 38 ETDRS letters. (B) to (D) Week 4, Month 3 and Day 10 post combined cataract and oil removal, the vision has recovered as the ELM appears intact (white arrows) whilst there is persistent discontinuity of the ellipsoid layer (red arrows) and persistent subfoveal fluid. (E) to (G) At months 6,9 and 12 there is gradual recovery of the ellipsoid layer (red arrows) with preserved clarity of the ELM (white arrows) and discrete hyper-reflections(blue arrow) in the ONL, the vision remains good and stable throughout

4.6.3.3.5 Patient 8

Figure 4.14: Preoperative SD-OCT of Patient 8

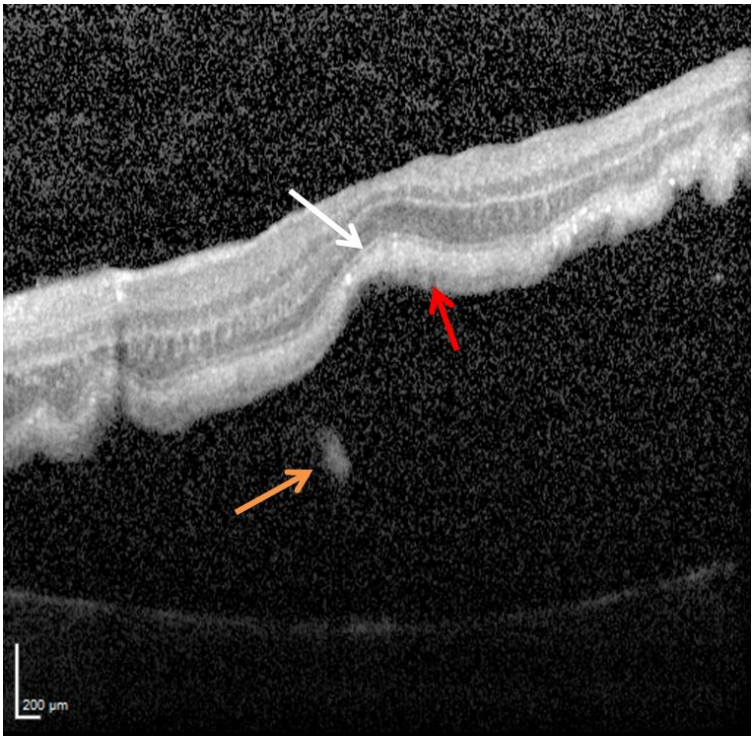
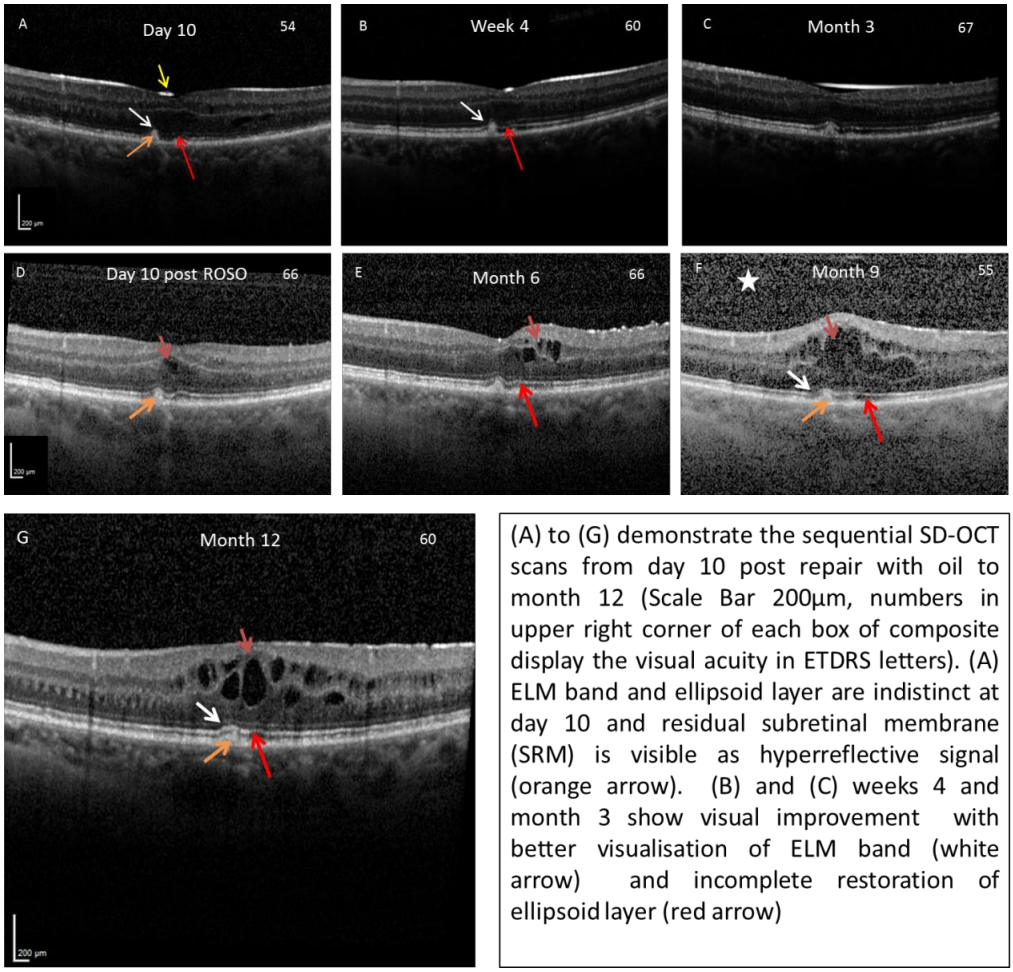


Figure 4.14 A 53 year old Caucasian male presented with an RD of 8 days duration and linear bands of subretinal PVR (Grade 4). Figure shows preoperative SD-OCT of detached fovea (scale bar 200µm). Accurate identification of outer retinal layers is limited but hyper-reflective band (white arrow) may represent ELM, thick undulating hyper-reflective layer may represent combination of inner and outer segments of photoreceptors, discrete hyper-reflective signal below detached retina (orange arrow) corresponds to subretinal PVR membrane. Presenting VA was 42 ETDRS letters.

Figure 4.15: SD-OCT Sequence of Patient 8



(A) to (G) demonstrate the sequential SD-OCT scans from day 10 post repair with oil to month 12 (Scale Bar 200µm, numbers in upper right corner of each box of composite display the visual acuity in ETDRS letters). (A) ELM band and ellipsoid layer are indistinct at day 10 and residual subretinal membrane (SRM) is visible as hyperreflective signal (orange arrow). (B) and (C) weeks 4 and month 3 show visual improvement with better visualisation of ELM band (white arrow) and incomplete restoration of ellipsoid layer (red arrow)

(D) 10 days post combined cataract and oil removal an intraretinal cyst (maroon arrow) is visible in the ONL, and the SRM (orange arrow) remains unchanged (E) at month 6 more cysts are visible within the inner layers of the retina (maroon arrow) , whilst there is preservation of the ELM and ellipsoid zone (red arrow) the vision remains stable (F) there are gross cystic changes throughout the retina at month 9 and the clarity of the ELM is reduced, the vision has worsened and note the increased intensity of pixels in the vitreous (white star) (G) at month 12 chronic intraretinal cysts persist, whilst the vision has improved along with clarity of the ELM (white arrow) and ellipsoid layer (red arrow). The SRM (orange arrow) appears unchanged.

4.6.4 Discussion

As expected, in comparison to baseline, vision improved upon successful reattachment of the retina. However, at day 10 post retinal reattachment, no evidence of outer retinal band restoration was observed in any of the eyes studied. Thereafter, by weeks 4 to 6, reorganisation of the ELM becomes noticeable which appears to correlate with visual recovery and supports the findings from section 4.5.10. In this series, ELM band resolution appears to occur either prior to, or concurrently with, ellipsoid layer restoration. This may suggest that the reorganisation of the photoreceptors post retinal reattachment occurs from inner regions, outwards.

The OCT scans of three patients demonstrated the presence of residual subfoveal fluid at Day 10 post RD repair. In all three of these patients, discontinuity of the ellipsoid layer was present but with preservation of the external limiting membrane. The sequence of scans outlining the postoperative course in patient 5, suggest that visually recovery can occur independently of persistent subfoveal fluid resorption. This confirms findings reported by Seo *et al* [303] but conflicts with Ricker *et al* [302]. Drawing direct comparisons is limited by the small numbers in our series and the differing operative techniques, as both of the aforementioned groups report on findings after scleral buckling surgery.

The significance of the integrity of the ELM requires further elaboration. Anatomically it represents the collective row of zonular adherentes connecting the apical processes of the Muller cells with the cell bodies of the photoreceptors [321, 322]. Wakabayashi *et al* postulated that ELM disruption represented an irreversible state of photoreceptor damage as complete restoration of the ellipsoid layer was not observed following ELM disruption. However, in this study we have observed poor ELM resolution at day 10 in most eyes, followed by subsequent reorganisation by week 4. Furthermore, we have noted that visual recovery can occur independently of complete ellipsoid layer restoration but that it does seem to correlate with ELM integrity in both the study of primary PVR eyes and in our cross sectional study.

The process of visual recovery in PVR eyes post RD is complex. Laboratory work from explanted peripheral human retinectomy specimens has demonstrated that most of the structural motifs necessary for visual recovery remain in place, even in advanced disease [45]. The pathological events in retinal detachment not complicated by PVR are therefore of central importance to understanding the reasons for the poor visual outcomes in PVR. Müller glia play a central role in the intraretinal remodelling response to retinal detachment. Changes in Müller cell processes are seen within 24 hours of experimental RD [323]. By day 3, the altered Müller cell processes which extend into the subretinal space, appear to do so through localised disruptions in the ELM. Those cells which extend into this region (rather than those which remain intraretinal) may ultimately form multi-layered scars [324]. Müller cell processes within the sub retinal space appear to inhibit photoreceptor regeneration [293]. Furthermore, since glial extensions have been demonstrated in excised epiretinal and subretinal PVR membranes, our findings of ELM disruption as a correlate to limited visual recovery are supported.

It could therefore be proposed that disruptions in the ELM which are visible on OCT, may not only represent more extensive damage to the photoreceptor cell bodies as previously thought [311], but may actually indicate that the equilibrium of the intra-retinal glial response has been shifted from protective to destructive. Furthermore, it is possible that the aforementioned OCT studies on eyes without PVR where ELM was found to correlate to visual recovery, may actually be describing a subtle sub-clinical demonstration of PVR in its early stages.

4.7 Summary

We have noted both in the multiple regression analysis of our cross sectional study and anecdotally in the longitudinal analysis of eyes with primary PVR, that vision appears to correlate with ELM integrity and this is supported by laboratory reports. Should our findings be validated, differentiating the different aspects of features we have observed may be important in the design of future trials of new treatment.

4.8 Grading Intraocular Inflammation

Analysis of intraocular inflammation may be performed clinically or using automated tools. The former is subjective. Its reliability may be observer dependent and thus may be inherently limited by subjectivity. Classification and standardisation of inflammation is important.

Proliferative vitreoretinopathy is an inflammatory condition and the work in this thesis aims to identify strategies to improve outcomes in these patients. Establishing an objective marker of inflammatory activity may not only facilitate 'real-life' disease management of PVR but may prove critical in the context of future clinical research, where clearly defined endpoints are required.

4.8.1 Clinical analysis of intraocular inflammation

Clinical methods of classifying aspects of intraocular inflammation involve quantifying the following parameters in the anterior and posterior chambers:

- anterior chamber cells
- anterior chamber flare
- vitreous haze

The most widely employed system in clinical practice was initially described in 1959 by Hogan, Kimura and Thygeson when classifying inflammation in eyes with uveitis [325]. The number of anterior chamber cells visualised in a 'wide beam with a narrow slit' were summed and categorised into one of four categories (i.e. 1+, 2+. 3+ or 4+) reflecting increasing severity of disease activity. Descriptions of anterior chamber flare had rather less well-defined classification criteria. Its severity was graded depending of the clarity by which intraocular structures (i.e. iris/lens) were visible [325].

Classification of vitreous haze was first formally described by Nussenblatt *et al* in 1985 [326]. In their report, they describe a similar incremental categorical scoring system whereby the observer views the fundus through an indirect binocular ophthalmoscope. The viewed image is then compared to standard photographs with varying degree of fundal clarity and feature obscuration. The inflammatory activity in the vitreous therefore ranges from the greatest amount (4+) through lesser intermediate points (3+, 2+, 1 +, trace) to no evident vitreous haze at all (0).

Where the optic nerve head is obscured the vitreous haze is graded as 4+. Where the optic nerve head is visible but with indistinct borders, the grade is 3+. A 2+ haze allows for better visualization of the retinal vessels, while 1 + permits a better definition of both the optic nerve head and the retinal vasculature [326] . The differences between trace vitreal haze and 0 are sometimes subtle. The trace eyes are those in which the nerve fibre layer striations cannot be visualized and there may be slight blurring of the optic disc margin.

In 2005, the Standardization of Uveitis Nomenclature (SUN) Working group published guidelines on the reporting of clinical data in eyes with uveitis [240] . In addition to standardising diagnostic terminology and outcome measures, group members agreed a consensus of grading intraocular inflammation in terms of the three aforementioned parameters above.

Table 4.5, Table 4.6, and Table 4.7 which follow display the SUN criteria for grading anterior chamber cells, anterior chamber flare and vitreous haze

Table 4.5: SUN Working Group Grading Scheme for Anterior Chamber Cells

Grade	Number of Cells in Field*
0	<1
0.5	1-5
1	6-15
2	16-25
3	26-50
4	>50

* Field Size is 1mm by 1mm Slit Beam

Table 4.6: SUN Working Group Grading Scheme for Anterior Chamber Flare

Grade	Description
0	None
1+	Faint
2+	Moderate (iris/lens details clear)
3+	Marked (iris/lens details hazy)
4+	Intense (fibrin/plastic aqueous)

Table 4.7: SUN Working Group Grading Scheme for Vitreous Haze

Grade	Description
0	None
0.5+	NFL striations not visible
1+	Clarity of Retinal Vasculature and Optic Nerve Head
2+	Retinal Vessels Visible
3+	Visible Optic Nerve Head (indistinct borders)
4+	Optic Nerve Head Obscured

NFL = Nerve Fibre Layer,

The SUN classification of vitreous haze mirrors that reported by Nussenblatt *et al* [326] with the simple substitution of ‘trace’ with ‘0.5’, for consistency.

4.8.2 Objective Measurement of Intraocular Inflammation

Although the SUN classification served to standardise nomenclature and facilitated the reproducible reporting of clinical signs, it remained subject to both inter and intraobserver variability [327]. Furthermore, it only provides a categorical incremental scale which at the lower end of disease severity is poorly discriminatory and lacks the sensitivity required in the clinical trial context [328, 329].

4.8.2.1 *Laser Flare-Cell Photometry*

Efforts to provide objective measurements of anterior chamber inflammation using automated techniques have principally relied on laser flare-cell photometry [330]. This technique quantifies aqueous humor protein (flare) and cells using either a helium-neon or diode laser that is projected into the anterior chamber [331, 332].

For flare measurement, light is detected by a photomultiplier which scans across a fixed area over a fixed time period (0.3mm X 0.5mm over 0.5 seconds). The average of two additional background readings above and below the fixed window are obtained. This is subtracted from the reading within the scanned window to provide the laser flare photometry measurement, which is expressed in photon units/msec [330].

Aqueous cells are detected similarly by a laser scan through a fixed aqueous volume over a fixed time period (0.5mm X 0.6mmX 0.25mm over 0.5 seconds). Light reflected off an aqueous particle is recorded as a peak of light by the photomultiplier and counted as a single cell (provided it is of an intensity higher than a preset background threshold). A fixed cell index (or count) is thus generated [330].

Laser flare-cell photometry has provided an objective measure of intraocular inflammation as determined by the degree of anterior chamber activity. It has been used to quantify intraocular inflammation various conditions involving both anterior and posterior segment pathology [333-337] . It is a safe and non-invasive technique that may be a useful research tool which provides a more accurate and reproducible method for quantifying anterior chamber protein and cells than clinical observation alone. Its precise role in clinical practice has yet to be definitively determined.

4.8.2.2 *Objective Measures of Vitreous Inflammation Analysis; VITAN*

Until recently, no objective measure of posterior inflammatory activity existed. Laser flare-cell photometry of the anterior chamber has been used as an indirect surrogate marker of posterior chamber inflammation but with obvious limitations.

In 2014, Keane *et al* published a pilot study describing their development of an OCT-derived signal of vitreous intensity in eyes with intermediate and posterior uveitis [338]. They describe an automated technique whereby the intensity of pixels calculated in a fixed compartment (the vitreous) can be summed to generate a mean OCT intensity. This is then expressed in arbitrary units as a ratio, in relation to the signal generated from the RPE (vit/RPE relative intensity ratio). This objective measure was found to correlate well to clinical scores of vitreous haze in uveitic eyes with and without haze, and normal controls.

A second report was published, the following year further validating the tool and described improvements to the automation process [339]. Where the initial pilot study required manual segmentation of the OCT to define the borders of the relevant compartments (RPE and vitreous), a fully automated algorithm was developed in order to eliminate observer subjectivity completely. Furthermore, additional indices were introduced as objective markers of vitreous inflammation.

I am grateful to Pearse Keane, Tariq Aslam and Alastair Denniston who have kindly provided me with the updated software (VITAN, vitreous) described in their recent publication in order to explore its role in the management of eyes with PVR.

4.8.2.3 *Aims and hypothesis*

The aims of the subsequent study were to determine the use of VITAN as an objective measure of vitreous inflammation in eyes which have undergone surgery with PVR in the context of work in this thesis:

As cystoid macular oedema is a well-defined primary endpoint, one might assume that the level of vitreous inflammation would be higher in eyes with this abnormality when compared to eyes without CMO.

Furthermore, eyes treated with a slow-release dexamethasone implant may be expected to display lower levels of vitreous inflammation than eyes without adjunctive corticosteroid.

Hypothesis:

1) There is a difference in the objective markers of vitreous inflammation in eyes with and without CMO, 6 months after successful retinal detachment surgery for PVR
2) Eyes treated with a slow-release dexamethasone implant have a lower 'marker' of vitreous inflammation after injection

4.8.3 Methods

4.8.3.1 *Selection of subjects:*

Of the 140 eyes in the Ozurdex in PVR Study, at 6 months post study vitrectomy, approximately one third retained silicone oil and hence were excluded. Forty five eyes with cystoid macular oedema and twenty seven eyes without CMO were included in the study. These eyes were not divided by treatment allocation, as the presence or absence of CMO was used as the dichotomising variable. Fifteen normal eyes served as controls.

For comparison of the proposed treatment effect of corticosteroid vs non-corticosteroid treated eyes, the time-point chosen for cross-sectional comparison was following the injection of the second Ozurdex® implant. At 10 days post combined silicone oil removal and implant injection, eyes were without internal tamponade. Furthermore, pharmacokinetic studies describe detectable differences in dexamethasone concentrations within the vitreous by day seven [213]. Twenty two eyes treated with Ozurdex and twenty four eyes without Ozurdex were compared. Fifteen normal eyes served as controls. The control data were kindly donated by Tariq Aslam and was obtained from a bank of normal subjects.

4.8.3.2 *Application of VITAN*

The OCT scanning protocol used has previously been described (Refer Section 3.2.4.5.5). B scans with the deepest foveal excavation were manually chosen for analysis. In eyes with cystoid macular oedema, or where a foveal dip was not visible, local anatomical landmarks were used to determine the scan through the fovea. Images were exported in a 1:1 pixel format with maximum resolution and zero compression. All scans were then opened in Windows Live Photo Gallery and cropped to a fixed dimension of 506 X 489 pixels.

VITAN is based upon the MATLAB image-processing platform (MathWorks, Natick, MA, USA), and was designed, developed, and coded to work with Heidelberg Spectralis OCT images [339]. MATLAB version R2015-b was used during this study.

4.8.3.3 *Deriving objective measures of vitreous inflammation*

The algorithm sequence was previously developed and validated by Keane *et al*. The process by which objective indices are obtained will be briefly outlined herein.

The OCT scan is loaded into the software and a sequence of algorithms initiated in order to automatically segment the image into binary form consisting of retinal and pigment epithelial layers. This then allows the software to precisely construct a rectangle consisting entirely of vitreous tissue, just anterior to the macula. Its position is confirmed by the user, or can be manually adjusted as necessary. Thereafter, the analysis proceeds as described above (section) generating a mean intensity signal relative to that derived from the segmented RPE layer. This allows for variation in image gain (e.g media opacity). Additional algorithms are derived using mathematical descriptions of texture relative to the RPE. Figure 4.16 displays this sequence.

Figure 4.16: Sequence of Automatic Segmentation in Vitreous Analysis

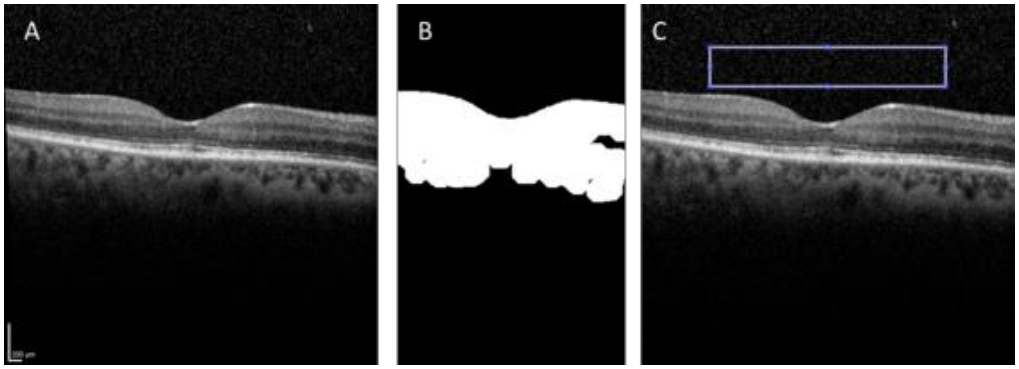


Figure 4.16 showing sequence of automated segmentation in order to automatically segment image to construct rectangle for analysis (A) initial B scan image uploaded into software (B) binary from of image following automatic segmentation (C) rectangle constructed consisting entirely of vitreous and sited anterior to macular. Mean intensity of pixels contained in this area is calculated

4.8.4 Data analysis and statistical methods

The two imaging process measures were a) the mean intensity adjusted for the RPE (MIA) and b) the texture intensity adjusted for RPE (TIA). These two objective indices were chosen as they are previously validated measures of vitreous inflammation. Statistical comparisons were made using the Kruskal Wallis H test and Mann Whitney U test for non-parametric data in IBM SPSS version 22.0.

4.8.5 Results

4.8.5.1 *Accuracy of automated vitreous segmentation*

A total of 133 scans were processed. Four scans were of insufficient quality for the software to initiate the algorithm sequence. The automated box placement was inaccurate in seven scans and required manually siting. Statistical analysis was performed both including and excluding these eyes.

4.8.5.2 *Adjusted mean intensity (MIA) and texture intensity (TIA) values; cystoid macula oedema*

The median MIA in eyes with cystoid macula oedema was 19.1, compared to 15.8 in eyes without. This difference was not statistically significant. However, the median MIA of both groups were significantly higher than he MIA in normal controls (MIA = 7.2, p=0.001).

The median TIA in eyes with CMO (TIA = 5.07) was also similar to eyes without CMO (TIA = 4.63) but significantly higher (p=0.001) than normal controls (TIA = 1.12). Figure 4.17 and Figure 4.18 display the box plots demonstrating the sample distributions for MIA and TIA by group.

Figure 4.17: Boxplot of Mean Intensity Adjusted for RPE (MIA) Score in PVR Eyes; CMO comparison

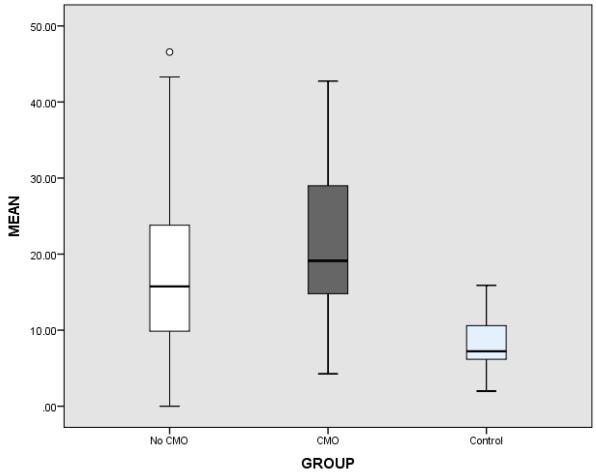


Figure 4.17 demonstrates the mean intensity adjusted for RPE (MIA) is comparable between eyes with and without CMO but significantly higher than normal controls (central bar = median, box = interquartile range, whiskers = range)

Figure 4.18: Boxplot of Texture Intensity Adjusted for RPE (TIA) Score in PVR Eyes; CMO comparison

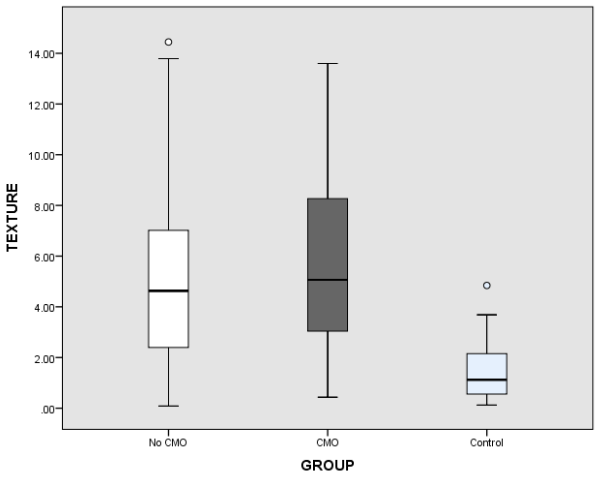


Figure 4.18 boxplot demonstrates the texture intensity adjusted for RPE (TIA) is comparable between eyes with and without CMO but significantly higher than normal controls (central bar = median, box = interquartile range, whiskers = range)

4.8.5.3 *Adjusted mean intensity (MIA) and* texture *intensity (TIA) values; day 10 post implant*

The median MIA in eyes day 10 post -implant was 21.50 compared to 19.41 in eyes that had not received the implant. This difference was not statistically significant. However, again the median MIA of both groups were significantly higher than the MIA in normal controls (MIA = 7.23, p<0.0001).

The median TIA in eyes treated with Ozurdex (4.58) was also similar to untreated eyes (5.23) but significantly higher (p=0.001) than normal controls (TIA = 1.12). Figure 4.19 displays the box plots for MIA and TIA by group.

Figure 4.19: Boxplots of Adjusted Mean and Texture Intensity Scores Day 10 Postoperatively

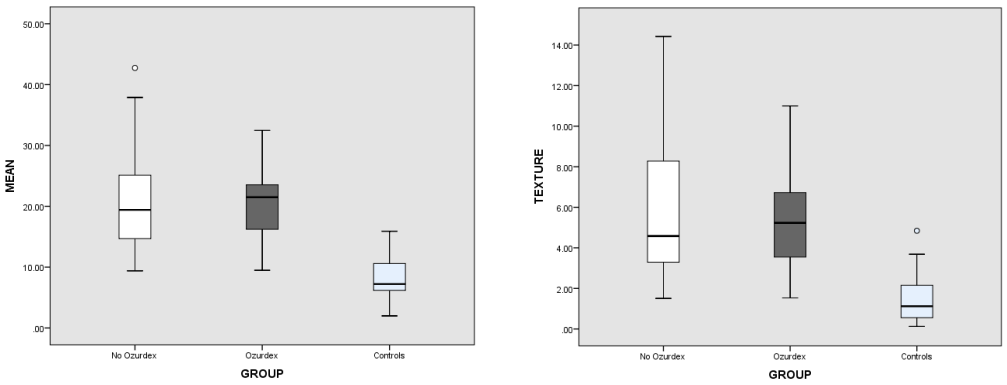


Figure 4.19 above demonstrates that mean intensity adjusted for RPE (MIA) and texture adjusted for RPE (TIA) are comparable between eyes treated and not treated with Ozurdex, but significantly higher than normal controls ((central bar = median, box = interquartile range, whiskers = range)

4.8.6 Discussion

This custom image analysis software (VITAN) has been so far validated as an objective tool to measure vitreous inflammation in eyes with uveitis [339]. Recently, the 1st generation automated tool adopting the vit/RPE index (which preceded VITAN) was shown to have comparable sensitivity between eyes irrespective of phakic status and previous vitrectomy [340].

The VITAN software was user friendly and only failed to initiate the automated algorithm sequence in four scans out of 133. It correctly segmented the images in order to generate the rectangular box for analysis in all bar 7 eyes (5.3%). In these eyes, the rectangle was manually positioned which upon sensitivity analysis, did not affect the overall findings.

Considering the two measures of inflammation, mean intensity adjusted for RPE (MIA) and texture intensity adjusted for RPE (TIA), we found no difference between the groups with and without CMO. However, there was a notable difference between these groups and the control population in both these indices.

Figure 4.20 shows examples of images in each group with their corresponding intensity scores.

Figure 4.20: Example of Varying Vitreous Intensity Scores

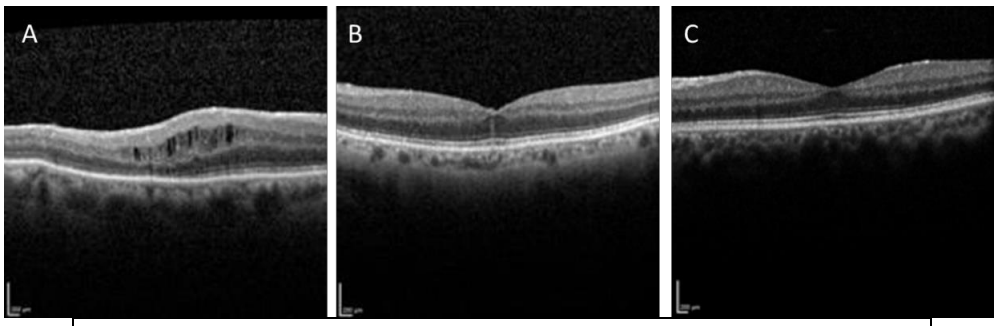


Figure 4.20 highlights examples of scans of eyes post PVR detachment surgery with and without cystoid macular oedema and normal controls. Their respective intensity scores are as follows (A) Eye showing CMO with a mean intensity adjusted for RPE (MIA) of 15.87 and texture intensity adjusted (TIA) score of 3.11, (B) No CMO where MIA is 11.89 and TIA is 1.99 (C) normal control eye where MIA is 4.12 and TIA is 0.54

PVR is an inflammatory process, and surgical intervention is intrinsically perceived as an injury, thereby further contributing to the inflammatory response. It is therefore unsurprising that these eyes 'score' higher in markers of vitreous inflammatory activity compared to normal eyes. Drawing comparisons to Keane *et al*'s publication, the MIA and TIA scores for both groups (CMO vs no CMO) are similar to those eyes which correlated to clinical scores of 2+ vitreous haze. Interestingly, the VITAN scores correlating to clinical vitreous haze scores of +1 and +2, are the least discriminatory of subgroup classification and may offer a reason as to why we did not observe a detectable difference between eyes with and without CMO.

Additionally, the TIA was found to correlate marginally better than MIA with clinical grades of vitreous haze in the report by Keane *et al*. They describe the image texture as that referring to 'spatial variation in pixel intensity [which] allows quantification of intuitive features such as roughness or bumpiness of the image'.

At 10 days after removal of oil, eyes with a slow–release dexamethasone implant did not show a significantly lower MIA or TIA compared to eyes which had undergone standard care. However, both groups scored higher than normal eyes. Pharmacokinetic studies have shown a detectible rise in dexamethasone concentration by day 7 post injection [213], but this does not seem to correlate to reductions in objective markers of inflammation in eyes with the implant *in situ* in our cohort. As the peak in dexamethasone concentration and secondary therapeutic action of Ozurdex peaks by month 2, analysis at this time point may increase the chances of VITAN detecting a difference between the two groups.

4.8.6.1 *Study Strengths and Limitations*

Observer bias is unlikely to be a significant limitation of this study as the process is near fully automated, with manual input required in only 5% of eyes. Masking of the primary assessor in eyes with and without CMO is not possible as it is visualised on the scan. Nevertheless, as the primary assessor, I was not masked to the treatment allocation of eyes which had received the dexamethasone implant and this must be acknowledged.

Our study has limitations which will be discussed in turn. Firstly, assumptions have been made that the clinical correlations of VITAN have already been validated and no further attempts were made to correlate our objective indices clinically. This was due to the retrospective design of this study. It is also important to note that the previously reported correlations of VITAN with clinical haze score were moderate [338], and discordances of agreement may reflect limitations of both.

Additionally, only one foveal B scan was selected for analysis. VITAN generates intensity indices relative to the RPE in order to compensate for overall variability in image gain. Nevertheless, to further account for this variability, analysing more than one B scan per eye and averaging the indices may be preferable.

The cross-sectional design of the study may have also limited our ability to detect differences in both groups of interest, particularly in the comparison between eyes with and without adjunctive Ozurdex. A longitudinal study investigating the change in VITAN indices may increase the sensitivity of detecting differences in vitreous inflammation in eyes where the treatment effect varies over time. This may also overcome the limitations of inter-eye variability that may exist.

4.8.7 Conclusion

PVR eyes have higher level of inflammation than normal and hence a higher likelihood of developing CMO and fibrogenic growth factors. The custom SD-OCT software VITAN may provide and objective measure of vitreous inflammation in eyes following surgery for PVR, but appears to lack the sensitivity required to discriminate between subtle differences at moderate levels of inflammation in our cohort. Further longitudinal studies in this population may be helpful to further develop the application of this objective tool.

4.8.8 Relevance of this work to thesis

The lack of observed treatment effect in the primary anatomical outcome of the Ozurdex in PVR study may in part be due to the optimistic nature of the primary endpoint. However, should VITAN prove to be a reliable discriminator of moderate or marked grades of inflammation, it may serve as an ancillary tool as an objective measure of disease activity in the clinical trial context. This may be of particular value in future PVR trials where modifying the inflammatory response and objectively assessing the level of blood ocular barrier breakdown is important.

5 Exploratory Investigation into Retinal Regenerative Strategies in Eyes with PVR

5.1 Müller Glial Cells

Müller cells are the principal glial cells found in the vertebrate retina and can be considered analogous to oligodendrocytes in the central nervous system. Müller cells span the entire depth of the retina. Proximally, their expanded endfeet lie adjacent to the internal limiting membrane and abut the vitreous body, with their distal limit marked by tight junctions with photoreceptor inner segments, to form the external limiting membrane [341]. They are, therefore, able to ensheath or interact with all retinal neuronal somata and processes and form both an anatomical and functional link between all compartments within the retinal microenvironment from the vitreous body proximally, to the choroidal circulation via the RPE, distally [342](Figure 5.1.)

Figure 5.1: Müller glial cell morphology

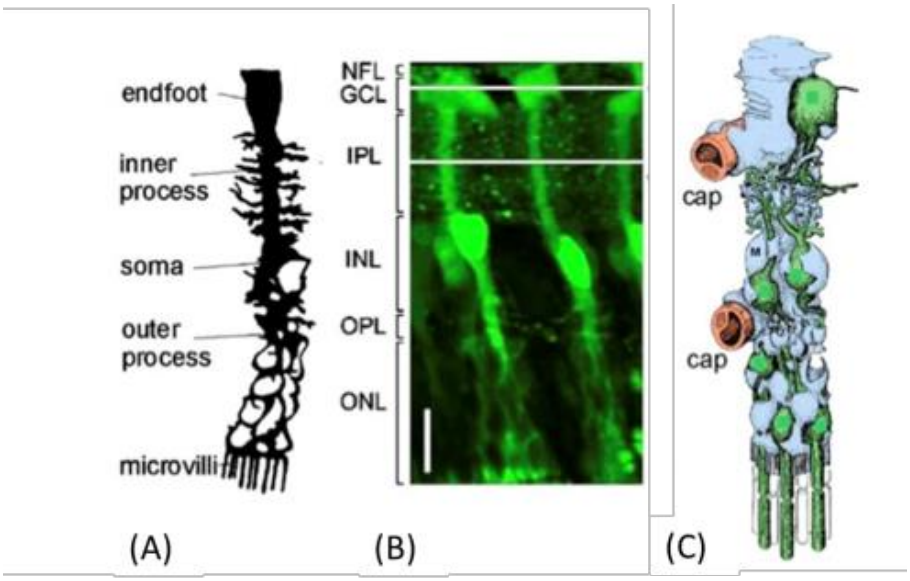


Figure 5.1 Müller glial cell morphology (Adapted from [342]with permission) (A) Drawing of a Golgi-labelled rabbit Müller glial cell (B) Müller cells of a guinea-pig in slice selectively stained with Mitotracker orange (green), (C) artist's impression of a human Müller cell (blue) enveloping various types of neurones (green) and interacting with retinal capillaries (cap, red) NFL = nerve fibre layer, GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL= outer plexiform layer, ONL =outer nuclear layer

5.1.1 Role of Müller cells

The role of the Müller cell in the vertebrate retina is diverse and extensive, performing the functions that oligodendrocytes, astrocytes and ependymal cells effect in other regions of the central nervous system [343]. In addition to stabilising the complex retinal architecture and providing neuronal support, they have key roles in the buffering of K^+ and water, free radical scavenging, neurotransmitter recycling and the regulation of retinal blood flow. Figure 5.2 and Table 5.1 summarise these key functions.

Figure 5.2: Müller cell interactions in healthy mature retina

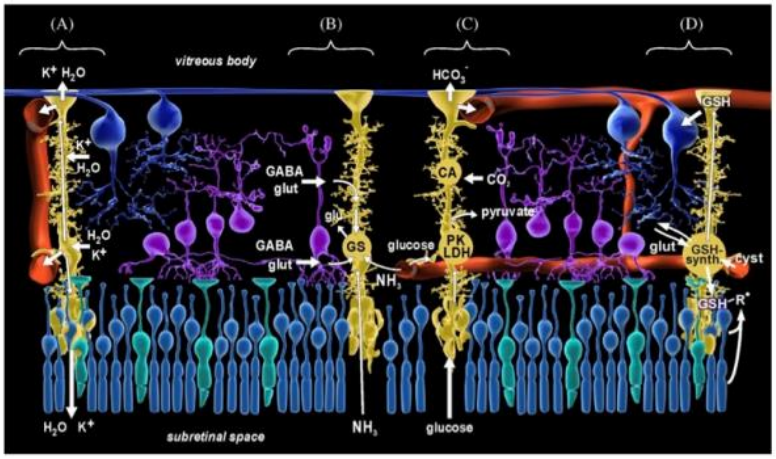


Figure 5.2 (Adapted from [342] with permission requested*)* Important Müller cell interactions in healthy mature retina (A) buffering of K^+ ions and water (B) neurotransmitter recycling (C) retinal blood flow regulation and maintenance of BRB (D) free radical scavenging/GSH metabolism (Key CA = carbonic anhydrase, cyst = cysteine, GABA = gamma-aminobutyric acid, glut = glutamate, GS = glutamate synthetase, GSH = glutathion, LDH = lactate dehydrogenase, PK = pyruvate kinase, R° = free radical molecule

5.1.2 Müller glia as stem cells

Stem cell-based therapies to replace irreversibly damaged tissue has proved successful in the anterior segment where access is good and the target structure is relatively simple [344]. However, regenerating the complex multilayered neural retina has highlighted many problems which remain unsolved.

Fish and amphibians regenerate neural retina throughout their life [345]. Compelling evidence for the ability of a population of Müller glial cells (MGC) to regenerate retinal neurons in the post-natal eye has been demonstrated in the adult zebra fish. In this species, Müller glia cells constitute the retinal stem cell niche and are able to regenerate the complex multilayered retina following full thickness injury. This ability for regeneration has been shown to be the result of Müller cell de-differentiation, re-entry into the cell cycle and production of a progeny that subsequently differentiates into all types of retinal neurons [346]. Earlier studies showed that Müller cells were capable of regenerating chick [347] and rat retina [348, 349] *in vitro* in early postnatal life.

The first report of successful isolation of an immortalised human Müller glial cell line (MIO-M1) was published in 2002 [350]. It was derived from the whole retina of a cadaveric specimen, from a 68 year old female donor. Since then, multiple cell lines have been generated from whole adult cadaveric retina and these, along with the MIO-M1 line, have subsequently been confirmed to exhibit neural stem cell characteristics [351]. Detailed characterisation of the distribution of these cells within the retina, have shown a higher population density towards the periphery [352]. More recently, human Müller glial cells (hMGC) have been shown to be a potential source of retinal ganglion cell precursors [353] and rod photoreceptors [354], the latter derived from MGCs retrieved from surgical adult explants.

Retinal diseases that lead to blindness are commonly associated with reactive Müller cell gliosis [355] and the release of inflammatory cytokines such as TGFβ, TNFα, IL-6 and IL-1 [356-359]. Furthermore, there is growing evidence to support the hypothesis that the intraretinal Müller cell response may be the key player in the development of PVR. It may also be a major contributor to visual impairment in eyes with restoration of grossly normal anatomy following successful retinal re-attachment (refer Chapter 4).

Since Müller stem cells are present in the adult human eye and *in vitro* they can be induced to differentiate into neurons, it would be expected that after disease or injury, these cells would be able to regenerate damaged retina and restore function such as that seen in the zebrafish. This prompts an investigation into why we observe a dwindling ability for the retina to regenerate following disease or injury as vertebrate evolution increases.

Retinal progenitor and Müller glial cell proliferation in the developing rat retina can be inhibited by co-culture with dissociated adult rodent retinal cells. This effect has been attributed to TGFβ signalling [360]. *In vivo* inhibition of TGFβ signalling resulted in potentiation of the EGF mediated proliferation of Müller glia [360]. Taking into account the effect of inflammatory cytokines on retinal gliosis, it is reasonable to hypothesize that the interaction of pathways involved in neurogenesis and inflammation may affect the ability of Müller glial stem cells to endogenously regenerate the diseased retina.

5.2 Research Question and Hypothesis

Since the human eye harbours stem cells with neurogenic ability, the isolation of these cells from peripheral biopsies of the retina during vitreoretinal surgery may provide a significant step towards developing cell based therapies for autologous transplantation. Furthermore, the stimulation of endogenous proliferation and neural differentiation to replace diseased neurons may be a preferable alternative to transplantation.

It is necessary to gain a better understanding of the factors that regulate and negatively control the ability of resident stem cells to regenerate the diseased retina. This project aims to identify factors that inhibit the proliferation of resident Müller stem cells within the adult human retina. In addition, it will examine whether blocking specific TGF receptors may reverse the inhibitory effects of this cytokine, known to be prevalent in the microenvironment of gliotic retina.

5.3 Objectives

1. To examine the feasibility of obtaining Müller glial cells with stem cell characteristics (hMSC) from retinal biopsies of patients undergoing vitrectomy and retinectomy in eyes with proliferative vitreoretinopathy (PVR)

2. To explore the role of TGFβ signalling on the growth and neural differentiation of hMSCs isolated from retinectomy specimens.

5.4 Methods

5.4.1 Acquisition of peripheral biopsies of adult donor human retina from surgical retinectomy specimens

The acquisition of human tissue for use in this study adhered to the tenets of the Declaration of Helsinki. Approval by the Research Management Committee at Moorfields Eye Hospital was gained and a favourable ethical opinion was granted by the National Research and Ethics Committee East Midlands - Derby. Following informed consent, retinectomy specimens normally discarded following surgery, were collected (as indicated in Figure 5.3 and Figure 5.4) from patients undergoing retinal detachment surgery complicated by proliferative vitreoretinopathy (PVR). Samples were collected in DMEM, transferred to the laboratory and processed within four hours of retrieval. The specimens were obtained either 1) as small fragments removed from the eye through a sclerotomy port using surgical forceps and/or 2) aspirated directly from the aspiration line of the vitrector during cutting. Both types of specimens were transferred immediately after retrieval into DMEM before processing.

Figure 5.3: Sequence of retinectomy retrieval using forceps

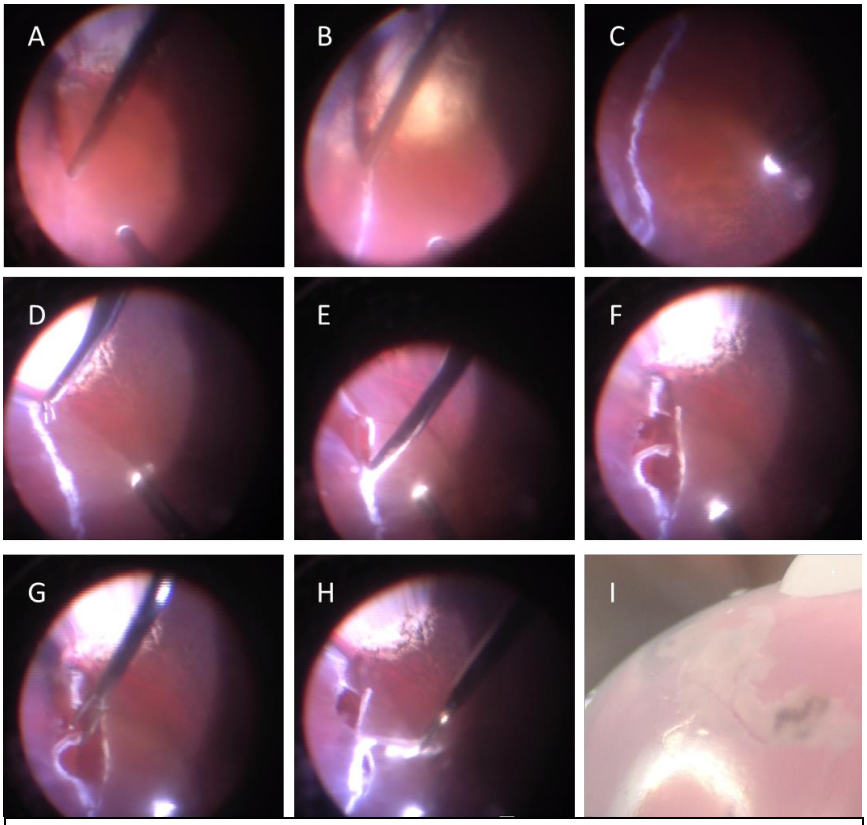


Figure 5.3 (A) to (C) Linear endodiathermy is applied to detached retina for haemostasis at site of proposed retinotomy, (D) to (E) angled micro-scissors are introduced to create linear retinotomy and advanced to desired length , (F) 'flap' of retina approximately 3mm x 1mm is fashioned and left on pedicle, (G) to (H) end-gripping micro-forceps are used to grasp flap and detach from pedicle (I) explanted retinectomy specimen to be transferred to eppendorf for processing

Figure 5.4: Sequence of harvesting retinectomy specimen via cutter

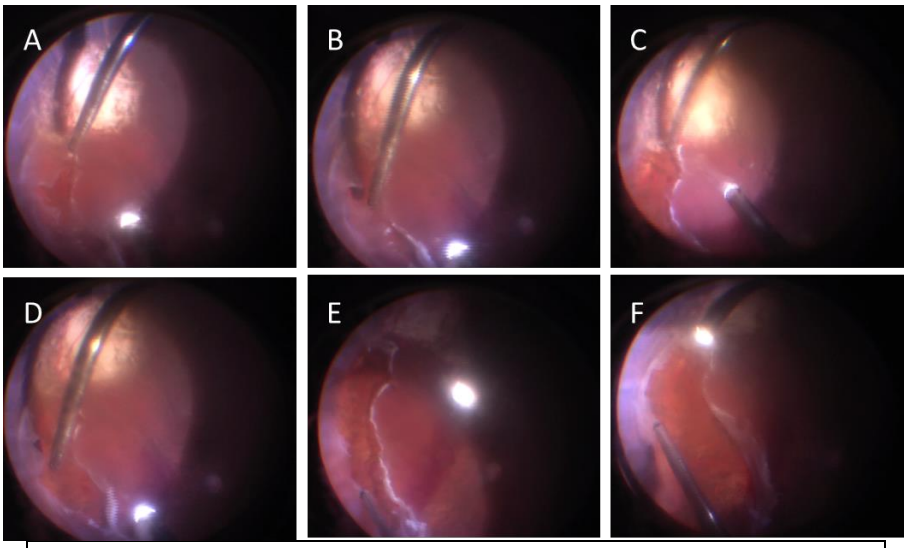


Figure 5.4 (A) Vitrectomy cutter is introduced into previous retinotom, adjacent to cut edge of site of retinectomy, (B) to (D) cutter is advanced along anterior edge of free 'redundant' retina whilst assistant gently aspirates syringe to retrieve sample in controlled fashion (care is taken such that the aspiration rate does not exceed the infusion to avoid the eye becoming hypotonous , (F) to (E) the residual anterior retina is removed, note the larger area of retinal tissue which is removed compared to small flap in Figure 5.3

5.4.2 Primary Cell Culture from retinal explants

Cell isolation was performed as previously described [350] with slight modifications to increase cell yield. Samples were received in serum-free DMEM with Penicillin and Streptomycin and centrifuged at 1400 rpm for 4 minutes. After discarding the supernatant, the tissue was re-suspended in 200µL TrypLE™ Express (1X) (#12604-013, Invitrogen) and incubated at 37ºC for 20 minutes. 500µL of DMEM with 10% foetal bovine serum was then added to inactivate the trypsin. Following 20-30 seconds of vigorous pipetting, the suspension was centrifuged at 1400rcf for 4 minutes. The supernatant was discarded and the pellet of cells resuspended in 2.4ml of media with 10% foetal bovine serum and epidermal growth factor (EGF) at a final concentration of 40ng/ml. Cell viability and initial cell counts were obtained after removing 24µL of this cell suspension and mixing with an equal volume of trypan blue. Cells were plated in central wells of a 24 well plate (4 well/sample) precoated with fibronectin (50 µg/ml; BD Bioscience, UK). The surrounding wells were filled with PBS in order maintain a moist environment. Plates were examined and photographed using a phase contrast microscope at regular intervals.

Cells were cultured for one week at 37°C in DMEM containing 10%FBS and 40ng/ml EGF with the medium replaced weekly thereafter. When cells reached confluence, they were detached using TrypLE™ Express (1X) (#12604-013, Invitrogen) for 3 min at 37°C and resuspended in fresh medium before re-plating in larger tissue culture plates.

5.4.2.1 *Modification to cell culture medium and addition of TGFβ signalling inhibition*

The aforementioned protocol was followed for the first ten explants, and subsequently modified as follows for six further specimens derived from the vitrectomy cutter. The primary cell culture medium of DMEM with 10% foetal bovine serum was substituted for DMEM/F12 (Invitrogen, Carlsbad, CA, USA, http://www.invitrogen.com/site/us/en/home.html) supplemented with 20 ng/ml bFGF ,4 mg/ml heparin , 0.1 mg/ml apo-transferrin, 25 mg/ml insulin, 1 mg/ml putrescine, 20 ng/ml progesterone,30 ng/ml sodium selenite, 1% BSA (Bovine Serum Albumin) (All Sigma-Aldrich, St. Louis, MO, USA, http://www.sigmaaldrich.com).

Cell pellets were re-suspended in the above neural stem cell medium with 40ng/ml of EGF, and samples divided into two (with and without TGFβ inhibition) before plating as above.

The following TGFβ inhibitory factors were added at the time of plating and at all subsequent media changes; TGF β RII – F_c (0.5μg/ml), SB-431542 (3ng/ml), TGFβ1,2,3 Ab (25μg/ml), (All R&D systems).

5.4.2.2 *Immunocytochemistry*

Immunocytochemistry was performed on: a) cells isolated from 1 donor sample at days 1, 4 and 7 in culture and b) retinectomy samples (approximately 3mm by 1mm) following cryosectioning from six live donors.

Cells were cultured on fibronectin-coated (50µg/ml) Lab-Tek glass chamber slides (Nunc, Inc, USA). After fixation with 4% paraformaldehyde in PBS, cells or cryosections of retinectomy specimens were blocked for 1 hour in 0.3% Triton-X in TBS, and incubated overnight at 4°C with primary antibodies diluted in the blocking solution. The following primary antibodies were used: Nestin (sc-21247; monoclonal; 1:100; Santa Cruz, USA. http://www.scbt.com/), SOX2 (AB5603; rabbit; 1:500; Merck Millipore, USA. http://www.millipore.com/), PAX6 (sc-11357; rabbit; 1:200; Santa Cruz, USA. http://www.scbt.com/), CRALBP (sc-28193; rabbit; 1:50; Santa Cruz, USA. http://www.scbt.com/), Vimentin (sc-5565; rabbit; 1:200; Santa Cruz, USA. http://www.scbt.com/) and GFAP (sc-9973; mouse; 1:200; Santa Cruz, USA. http://www.scbt.com/). Cells from the MIO – M1 cell line were used as positive controls. Mouse and rabbit IgG isotypes matching test antibodies were used as negative controls. After incubation with primary antibodies, specimens were washed in TBS, and then incubated for 3 hours with Alexa-conjugated secondary antibodies (Invitrogen, UK) at room temperature. Slides were washed and counter-stained with 2µg/ml 4',6'-diamino-2-phenylindole (DAPI) for 2 minutes and mounted with Vectashield mounting medium (Vector Labs, USA). Fluorescent images were recorded using a Zeiss LSM 710 confocal microscope operating in multitrack mode for FITC, DAPI and TRITC fluorochromes.

5.5 Results

5.5.1 Isolation of Müller glia with stem cell characteristics from retinectomy specimens

Ten retinectomy specimens from nine patients were initially studied without TGFβ inhibition, and a further six specimens were treated with TGFβ inhibitory factors. The typical dimensions were 3mm x 1 mm when retrieved directly with forceps. (Figure 5.3) The area of tissue retrieved by the vitrectomy cutter was difficult to measure accurately but was likely to be larger than that achieved using forceps (Figure 5.4).

After one week in culture, cells with the expected morphology of human hMSCs were observed in all processed samples, with higher yields achieved in samples derived from the cutter. They appeared bright when viewed under a phase objective and showed characteristic glial morphology. Considering the first ten samples, cells appeared to proliferate and form colonies by week 1. The majority, however, survived up to 8 weeks and appeared to form neurospheres (Figure 5.5) Attempts to release cells from these spheres and encourage proliferation were unsuccessful.

However, two cutter-derived samples appeared to proliferate and survived up to four passages and expansions into larger plates (Figure 5.6). Unfortunately, immortality was not achieved as eventually all cells were observed to adopt the morphology of terminally differentiated mature Müller glia with a characteristic flattened, enlarged and elongated cell morphology. One sample derived an additional cell preparation exhibiting a different morphology to that expected of hMSCs. These cells appeared epithelial in appearance, although they were not formally characterised by immunocytochemical staining (Figure 5.7)

Figure 5.5: Primary Cell Culture of Explants; colonies and neurospheres

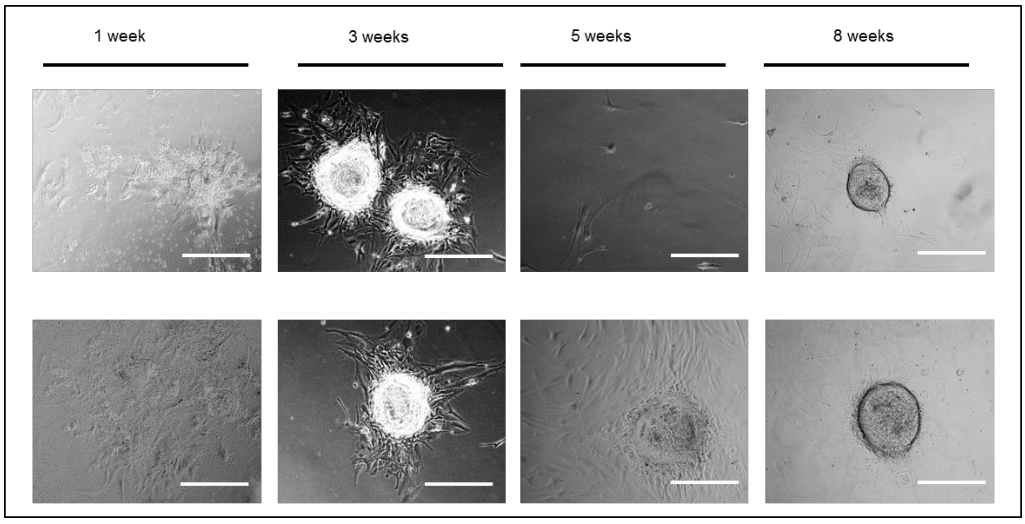


Figure 5.5 An example of cellular proliferation and colony formation by week 1 in a cutter derived sample. Note the characteristic morphology of hMGC and their bright appearance under a phase microscope. Formation of neurospheres was observed by week 3 with surrounding colonies of hMGC, attempts to release cells from spheres through enzymatic disruption were unsuccessful at both 5 and 8 weeks with no further proliferative activity observed – (scale bar 100µm)

Figure 5.6: Primary Cell Culture; proliferation to confluence

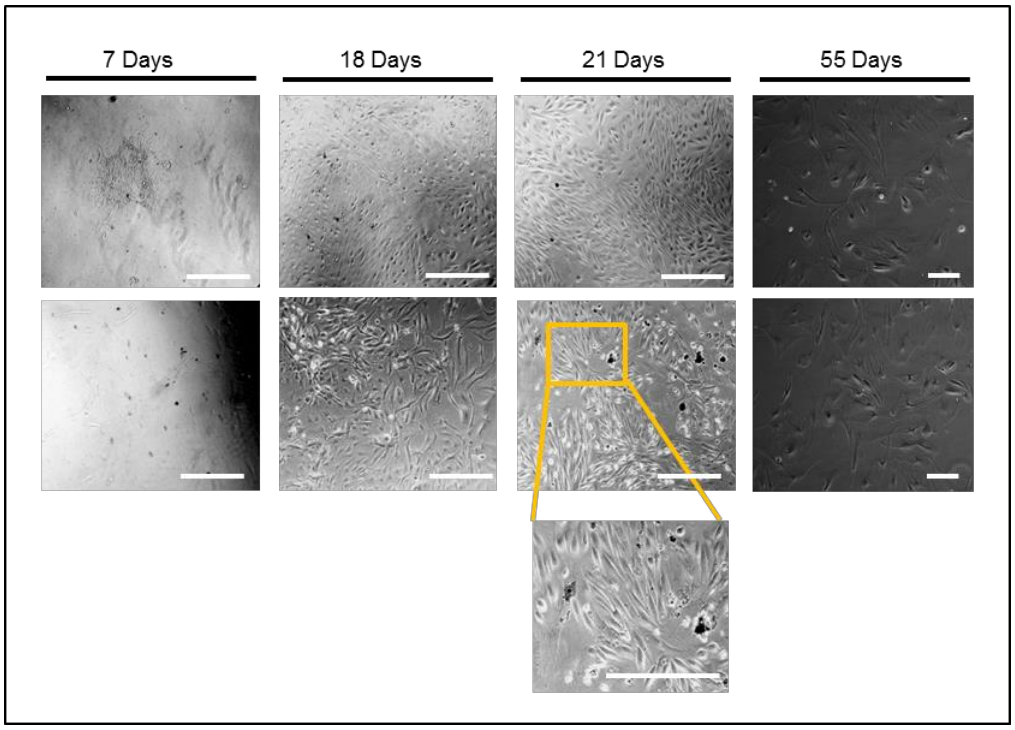


Figure 5.6 An example of cellular proliferation and colony formation by week 1 in a cutter derived sample. Cells reached confluence by week 3 and were expanded into larger flasks. By 7-8 weeks, cells appear flattened and adopted a terminally differentiated morphology (scale bar 100µm)

Figure 5.7: Primary Cell Culture; non hMGC morphology proliferative cells

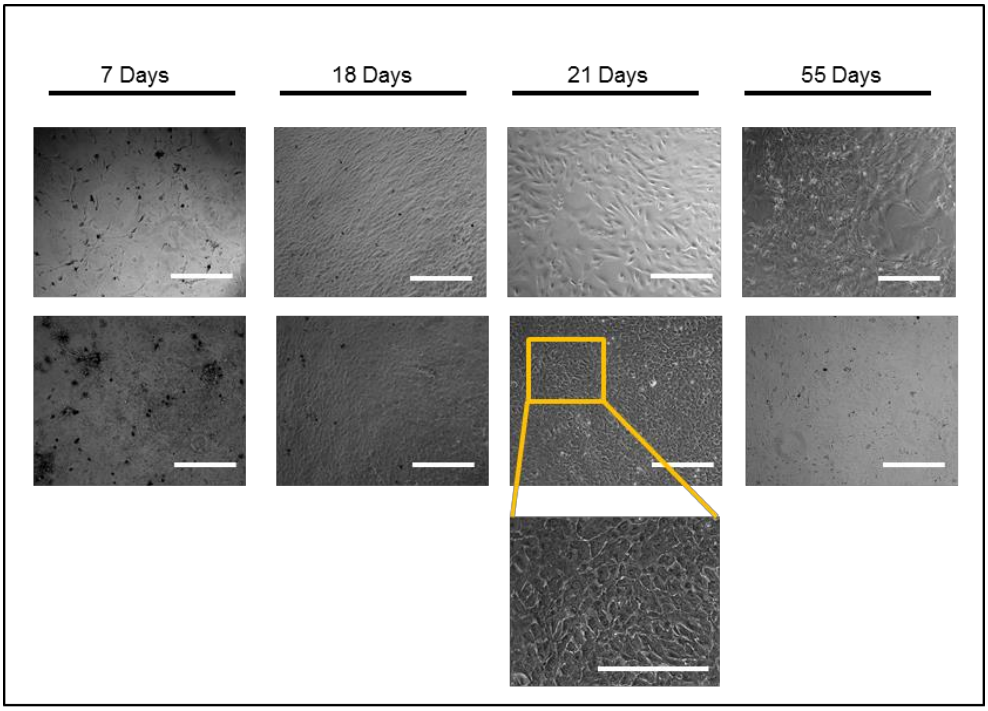


Figure 5.7 Cells of an epithelial morphology were isolated in addition to those with Müller glial stem cell morphology; at day 7 note how the wells contained extracellular pigment, at 3 weeks the cells appeared different in morphology to hMGC (bottom row) and appeared hexagonal, by 2 months very few cells with hexagonal morphology remained in culture (bottom row), whilst those presumed hMGC adopted a flattened and terminal morphology - scale bar 100µm

5.5.2 Isolation of Müller glia with stem cell characteristics from retinectomy specimens with TGFβ inhibitory factors

Considering the final six samples with modified culture media and TGFβ inhibition; again after one week in culture cells appeared to initially proliferate and form colonies. There did not appear to be any apparent difference in the cell yield when comparing those with additional TGFβ inhibition, and no samples reached confluence nor progressed to expansion into larger plates. Cells appeared to accumulate into neurospheres, and again attempts to release cells and encourage proliferation were unsuccessful. Eventually, they adopted the morphology of terminally differentiated mature Müller glia.

5.5.3 Characterisation of Müller glia with stem cell characteristics in culture from one donor sample

Cells harvested by both forceps and cutter retrieval methods from one adult donor were stained for the neuroprogenitor marker, Nestin, and the marker, CRALBP, which is expressed by Müller glial cells. Cells were removed from culture at day one, four and seven after primary plating. Figure 5.8 shows a cell staining positive for Nestin on day one. By day four a colony of cells were observed staining positive for both Nestin and CRALBP, and were noted to form a neurosphere at day seven (Figure 5.9).

Figure 5.8: Immunocytochemistry of cells in culture at day 1

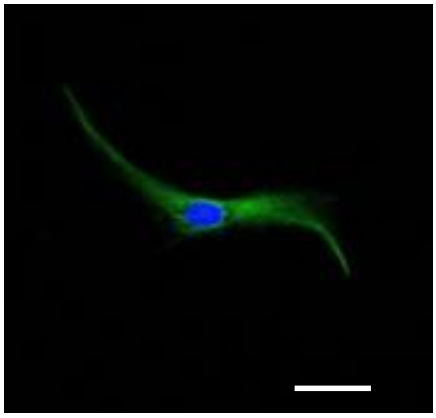


Figure 5.8 shows a single cell in culture at day 1 staining positive (green) for the neuroprogenitor marker Nestin. Central staining of nucleus (blue) with DAPI. The cell adopts the classic morphology of a human Müller glial cell with stem cell characteristics (hMSC). The cell did not stain positive for CRALBP.

Figure 5.9: Immunocytochemistry of Cells in culture at Days 4 and 7

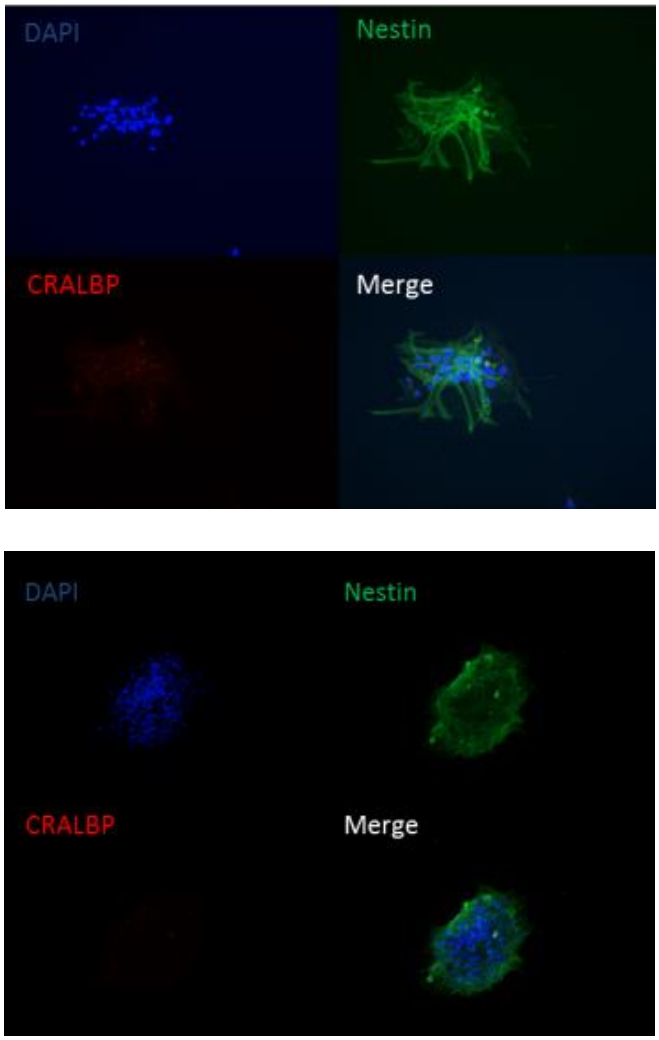


Figure 5.9 Immunohistochemistry composite shows a colony of cells in culture at day 4 (top) and day 7 (bottom), At day 4 the cells form a colony and stain positive for the neuroprogenitor marker nestin (green) and the glial cell marker CRALBP (red). (Nuclei stain blue with DAPI). At day 7, the cells appear to form a sphere and stain positive for nestin and weakly for CRALBP

5.5.4 Immunostaining of human retinectomy sections for neural progenitor markers and cell specific proteins

Retinectomy specimens from four adult donors were fixed in 4% PFA and stained for markers as mentioned above. The images shown in Figs 5.4.2, 5.4.3 and 5.4.4 are derived from cryosections of 15µm from one donor sample.

Most retinal sections appeared dystrophic and thickened but preservation of the overall retinal architecture was observed, with 3 distinct nuclear layers visible (stained positive with DAPI). In some sections, there appeared to be separation of the ganglion cell layer from the underlying outer retinal layers. This may represent trauma at the time of tissue retrieval, or damage during processing of the sample. Specimens stained positive for Nestin and CRALBP throughout the entire depth of the retina, with increased fluorescence observed at the inner retinal surface. Co -localisation was noted for both antibodies. Positive staining and co-localization was observed for GFAP and Vimentin. Non-specific staining was observed with PAX6 and samples did not show positivity for SOX 2. Positive controls confirmed activity of the primary and secondary antibodies.

Figure 5.10: Immunocytochemistry; positive control

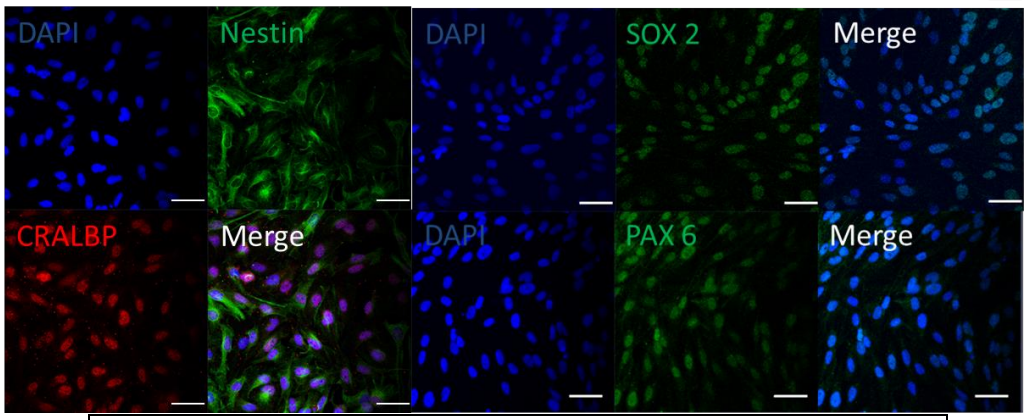


Figure 5.10 Composite figure of positive control staining for primary and secondary antibodies of cells from MIO-M1 cell line DAPI (blue nuclei), Nestin, SOX 2 and PAX 6 (green) and CRALBP (red) (Scale Bar 50µm)

Figure 5.11: Immunohistochemistry of retinectomy specimen; nestin and CRALBP

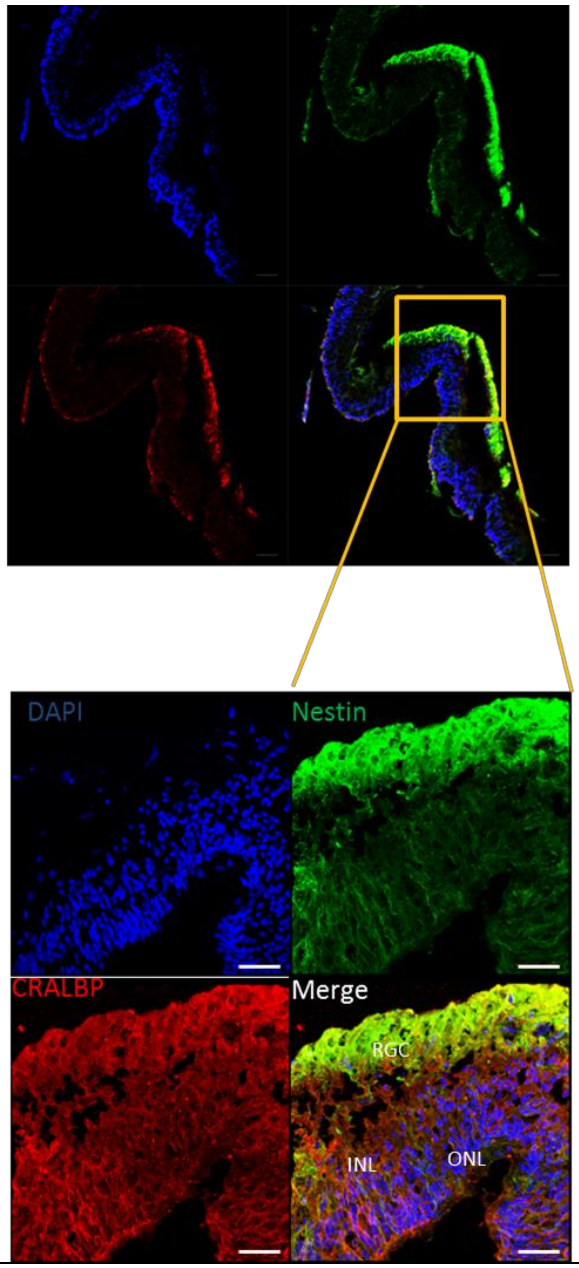


Figure 5.11 Immunostaining of retinectomy specimen as viewed under Zeiss 710 confocal microscope stained for DAPI (blue), nestin (green) and CRALBP (red) ONL, outer nuclear layer, INL inner nuclear layer, RGC, retinal gangliom cell layer (A) X10 magnification - Nestin and CRALBP were observed throughout the whole retinal thickness with co-localisation and greatest staining observed in inner retina (scale bar - 100µm (B) X40 magnification showing a high powered view of regions of increased staining in the inner retina, images also display thickened and dystrophic retina (scale bar 50µm)

Figure 5.12: Immunohistochemistry of retinectomy specimen; Vimentin and GFAP

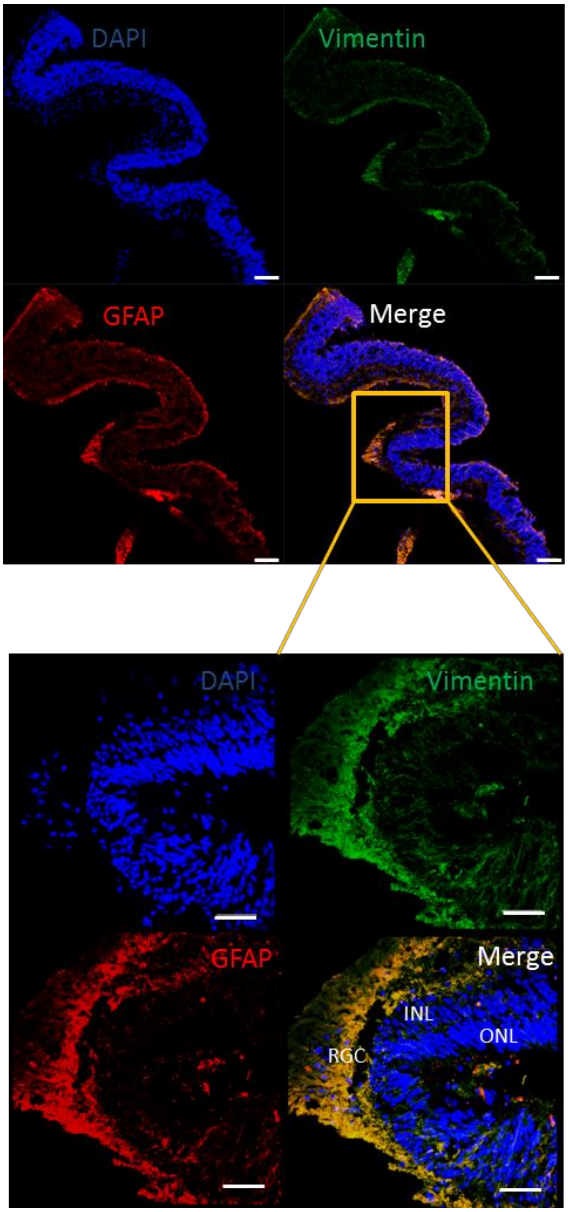


Figure 5.12 Immunostaining of retinectomy specimen as viewed under Zeiss 710 confocal microscope stained with DAPI (blue) , vimentin (green) and GFAP (red) ONL, outer nuclear layer, INL, inner nuclear layer, RGC, retinal ganglion cell layer (A) X10 magnification showing a marked staining for GFAP, indicating reactive gliosis with co-localisation of vimentin, a Müller glial cell marker (scale bar - 100µm (B) X40 magnification showing a high powered view of regions of increased staining in the inner retina. Images again demonstrate thickened and dystrophic retina (scale bar 50µm)

Figure 5.13: Immunohistochemistry of retinectomy specimen; PAX 6

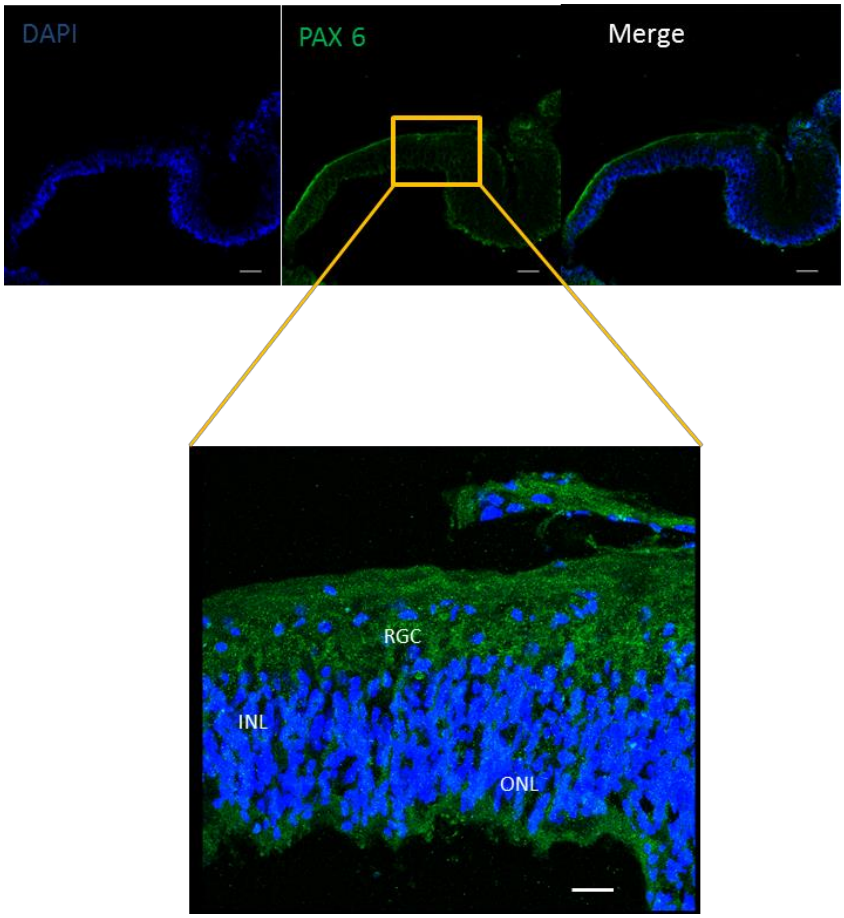


Figure 5.13 Immunostaining of retinectomy specimen as viewed under Zeiss 710 confocal microscope stained with PAX 6 (green) and DAPI (blue). ONL, outer nuclear layer, INL, inner nuclear layer, GCL, ganglion cell layer (A) X10 magnification – Non-specific staining observed in the inner retinal surface contrary to expected region of staining of this protein expressed in the nucleus (scale bar - 100µm (B) X40 Objective – higher powered images are inconclusive of positive staining for nuclei at inner nuclear layer (scale bar 50µm)

5.6 Discussion

Using established protocols, it was possible to isolate cells with the appropriate morphology of hMSCs which survived up to ten weeks in culture. However, only two of the sixteen specimens processed proliferated to reach confluence and were expanded into larger tissue culture plates. Successful hMSC culture from surgical specimens have reported initial cell counts of less than 10^6 yielding a 40% efficiency rate. This increases to 77% with higher cell counts (2 X 10^6) [354]. As the samples retrieved in this study are small fragmented pieces of tissue, it is difficult to achieve such high cell numbers. This may explain the lack of success in this study to date, and is consistent with the observed higher cell yield with cutter-derived samples when compared to those retrieved with forceps.

When specimens were cultured with the addition of TGFβ inhibitory factors, there was no effect on increasing cell yield, with an apparent overall reduction in proliferation seen. No samples proliferated to confluence when treated with TGFβ inhibitory factors. Previous reports in rodents describe a cytostatic effect of mature retinal neurons attributed to the effect of TGFβ signalling. Inhibition through a combination of TGFβRII – F_c protein and a pan TGFβ blocking antibody has been shown to enhance the ability of Müller glia to re-enter the cell cycle in response to EGF [360]. The lack of effect observed in this study may be also explained by the low initial cell counts at the time of plate seeding. Additionally, the cocktail of inhibitory factors used included a small molecule inhibitor of TGFβRI (SB-431542). In effect, with the combined inhibition of TGFβRI and TGFβRII – F_c, the entire TGF pathway may have been inhibited, thereby resulting in an 'excessive inhibition' of proliferation *per se*, with a lack of selectivity. Therefore, selective inhibition of TGFRs may yield different effects on cell proliferation and it may benefit further investigation.

All retinectomy samples were retrieved from eyes with proliferative vitreoretinopathy. Glial cell activation and reactive gliosis is a key feature in PVR, confirmed by the

marked staining for GFAP noted in the specimens in this study. Since it is likely that a large proportion of the resident glial cell population had already become activated and terminally differentiated towards reactive gliosis, it is logical to assume that the population of cells with stem cell ability may have already been depleted in this tissue. This may explain the negative staining for SOX2 and the non-specific staining for PAX6. Nevertheless, the positive staining for the neuroprogenitor marker, Nestin, both in retrieved retinectomy specimens and in cells early in culture suggest that the population of Müller glia with stem cell characteristics may not be completely absent in this dystrophic tissue.

As two samples initially showed signs of proliferation in which cell growth arrested at approximately seven to eight weeks was observed, future work may involve the study of local factors which inhibit proliferation at this stage. Sequential western blot analysis of the cells in culture, or proteomics of the culture media at specific time points may help to identify the changes in protein production which occur through an initial proliferative period, followed by a rapid cessation and terminal differentiation.

A proportion of the retinectomy samples studied were derived from patients enrolled in the Ozurdex in PVR Study. However, as the timing of sample retrieval was at the primary study vitrectomy, none of the eyes had undergone previous treatment with intravitreal corticosteroid. Further work may involve repeating the immunohistochemical analysis performed in this study with the addition of western blot analysis. A comparison between the expression of markers of reactive gliosis, and markers of neurogenesis in eyes treated with and without prior glucocorticoid treatment may be valuable. Whilst Kuo *et al* [72], in their rabbit model, observed neither a difference in the clinical manifestations of PVR severity, nor in GFAP expression in Ozurdex-treated eyes, it may be interesting to investigate this in human retinectomy specimens.

Similarly, it may then be interesting to reassess the feasibility of acquiring Müller glia with stem cell characteristics within the two treatment groups. It follows that if we were to find a reduction in reactive gliosis following the treatment of corticosteroid, we may benefit from a higher yield of cells with potential for immortality in the initial primary cell culture.

Ideally, peripheral biopsies from human retina would be large enough to obtain sufficient primary cell counts and 'healthy' enough to harbour a significant population of Müller glia with stem cell characteristics. In practice, this is difficult to achieve as patients undergoing vitreoretinal surgery, have by definition, vitreoretinal disease. Higher yields may be obtained from anterior retinectomies from large giant retinal tears of recent onset, thereby achieving adequate initial cell counts from tissue where reactive gliosis is less marked.

6 General Discussions and Future Work

This work has aimed to identify and test strategies focussed on improving the visual and surgical outcomes in proliferative vitreoretinopathy. During the process there were significant challenges in the clinical and laboratory investigations and much was learned both about the condition and how to investigate it.

6.1 Challenges

As with any clinical and scientific research, logistical challenges have presented themselves, some of which have been unpredictable. The process of gaining regulatory body approval for a clinical trial of investigational medicinal product is both rigorous and humbling. Ethical review board submissions, re-submissions and meeting attendances can be daunting but satisfying upon gaining a favourable opinion.

Obtaining an authorisation to conduct a clinical trial is a privilege. Whilst the process was complex and we were presented with unexpected new safety information shortly before submission, we were able to overcome these logistical challenges to gain authorisation within a self-imposed timescale. The novel learning experience throughout this process has already served me well for projects which have developed from work in this thesis, and will continue to do so in my future endeavours in clinical research.

Proliferative vitreoretinopathy is as complex a condition to manage clinically as it is to understand scientifically. A substantial proportion of data collection for this work was generated from the clinical management of 40 patients with a history of ocular trauma and 140 patients with established PVR. Cumulatively, I have estimated that over both study periods, I personally performed between 3000 – 3500 outpatient attendances clinical assessments and was present at over 400 operative procedures. This significantly tested my skills of time management, and may explain why recruitment rates are compromised when study teams perform multiple roles.

The challenges of preparing both oneself and one's study team for the level of scrutiny of an MHRA inspection should not be underestimated. Whilst we prided ourselves in the high level of safe and effective clinical and pastoral care we provided to all our

study participants, the uncertainty of whether this would be evident when subjected to external critique was a source of stress. Furthermore, the timing of the inspection fell at a critical period during my research fellowship and added to the challenges which I was already facing at the time.

Managing personal expectations of the success of laboratory work is another challenge. I have learned that despite one's best efforts, primary cell culture is unpredictable, cryo-sectioning can be monotonous but therapeutic, and immunohistochemistry is an art.

6.2 Successes

In some ways, any of the aforementioned challenges could comfortably position themselves in this subsection of successes. In order to complete the work required to write this thesis, these challenges were overcome. The work in this thesis has answered many research questions by proving and disproving hypotheses.

Ocular trauma is a blinding condition and has a propensity to vitreoretinal scarring. Whilst triamcinolone acetonide may not improve the anatomical outcomes in eyes undergoing vitreoretinal surgery following OGI, visual outcomes may be better in steroid-treated eyes. It could be argued that the AOT trial exceeded its study objectives. It confirmed the feasibility of an RCT in this disease group and provided sufficient data to power a large scale definitive study. Indeed, the findings of this trial were instrumental in the successful procurement of a £1000, 000 NIHR grant to conduct the study (ASCOT) of which the AOT trial was designed to test the feasibility.

The ASCOT Study forms part of the 'future work' which has already begun and its findings may help to definitively clarify whether triamcinolone plays a significant role in the management of open globe trauma.

Furthermore, as principal investigator at the primary ASCOT Study site and a co-investigator in the trial management group, I have forged valuable collaborations with

the Clinical Trials Unit at Kings College London. Established collaborations with many of the other 25 site PIs in the U.K will also prove a valuable resource for my involvement in multi-centre studies in the future.

The Ozurdex in PVR trial was the first study to investigate a slow-release dexamethasone implant in eyes with PVR. We successfully achieved our study aim by recruiting to time, maintaining retention of 99% of participants to the primary endpoint whilst securing a robust dataset. Despite a lack of efficacy in anatomical outcomes, in exploratory sub-analysis, we observed a potential treatment effect in terms of visual outcomes and rates of cystoid macular oedema. Further work investigating vision as a primary outcome measure may help to definitively answer the question. Future studies may require a multi-centre approach to achieve the desired sample size to maintain power to detect a statistically and clinically meaningful difference.

OCT guided analysis of the outer retina revealed the ELM as a correlate to visual recovery in eyes with PVR. This may add to the growing evidence that the intraretinal glial response plays a significantly larger role both in the development of PVR and as a vision limiting factor following successful RD repair. When published, this will be the first study to report these findings in this cohort. A larger study on a more homogenous group may help to validate our findings and serve to differentiate the different aspects of features we have observed. This may prove to be important in the design of future trials of new treatment.

OCT-derived indices of objective measures of inflammation are higher in eyes with PVR when compared to normal controls. If the sensitivity of discriminating higher levels of vitreous inflammation is improved, these indices may prove to be useful tools in establishing well-defined endpoints in future PVR studies.

The laboratory work outlined in this thesis constituted a small but important contribution to this endeavour. I was able to acquire skills in primary cell culture, cryo-

sectioning of tissue and immunocytochemistry. We isolated cells early in culture staining positive for nestin, a marker of neurogenesis and CRALBP, a glial cell marker. This suggests that a resident population of Müller glial cells with stem cell characteristics may exist in anterior dystrophic tissue with advanced proliferative vitreoretinopathy. Further work in primary cell culture, identifying the changes in protein expression which occur through an initial proliferative period, followed by a rapid cessation and terminal differentiation may prove valuable.

6.3 Summary

Studying disease in its advanced stage carries inherent disadvantages. The work in this thesis has suggested that it is difficult to modify the vitreoretinal scarring response sufficiently to improve anatomical outcome measures. For now, we may be better placed to improve the secondary causes of visual loss in eyes with PVR by focussing on vision as a primary outcome measure. This may be a plausible design for future vitreoretinal clinical trials in PVR. I hope to be a part of them.

7 References

1. John Forrester, A.D., Paul McMenamin, William Lee, *The Eye: Basic Sciences in Practice*, M. Parkinson, Editor. 2002, Saunders. p. 15.
2. *Scientific illustration for the research scientist*. Available from: www.somersault1824.com
3. John Forrester, A.D., Paul McMenamin, William Lee, in *The Eye Basic Sciences in Clincal Practice*, M. Parkinson, Editor. 2002. p. 37 - 44.
4. Available from: arthursclipart.org/medical/senseorgans/page_02.htm.
5. Michael Landers, L.H., *Retina*, in *Retina*. 1989, C.V Mosby Company. p. 105-109.
6. Mitry, D., et al., *Pathogenesis of rhegmatogenous retinal detachment: predisposing anatomy and cell biology.* Retina, 2010. **30**(10): p. 1561-72.
7. Larsson, L. and S. Osterlin, *Posterior vitreous detachment. A combined clinical and physiochemical study.* Graefes Arch Clin Exp Ophthalmol, 1985. **223**(2): p. 92-5.
8. Akiba, J., N. Ueno, and B. Chakrabarti, *Molecular mechanisms of posterior vitreous detachment.* Graefes Arch Clin Exp Ophthalmol, 1993. **231**(7): p. 408-12.
9. Bishop, P.N., et al., *Age-related changes on the surface of vitreous collagen fibrils.* Invest Ophthalmol Vis Sci, 2004. **45**(4): p. 1041-6.
10. Foos, R.Y. and N.C. Wheeler, *Vitreoretinal juncture. Synchysis senilis and posterior vitreous detachment.* Ophthalmology, 1982. **89**(12): p. 1502-12.
11. Wickham, L., et al., *Randomized controlled trial of combined 5-Fluorouracil and low-molecular-weight heparin in the management of unselected rhegmatogenous retinal detachments undergoing primary vitrectomy.* Ophthalmology, 2007. **114**(4): p. 698-704.
12. *The classification of retinal detachment with proliferative vitreoretinopathy.* Ophthalmology, 1983. **90**(2): p. 121-5.
13. Charteris, D.G., et al., *Proliferative vitreoretinopathy-developments in adjunctive treatment and retinal pathology.* Eye (Lond), 2002. **16**(4): p. 369-74.
14. Haller, J.A., et al., *Randomized controlled trial of an intravitreous dexamethasone drug delivery system in patients with diabetic macular edema.* Arch Ophthalmol, 2010. **128**(3): p. 289-96.
15. Machemer, R., *Massive periretinal proliferation (MPP) I. Pigment epithelium proliferation.* Mod Probl Ophthalmol, 1975. **15**: p. 227.
16. Machemer, R., et al., *An updated classification of retinal detachment with proliferative vitreoretinopathy.* Am J Ophthalmol, 1991. **112**(2): p. 159-65.
17. Banerjee PJ, C.D., *Retinal Redetachment Due to Proliferative Vitreoretinopathy* in *Complications of Vitreoretinal Surgery*, W.D. lois N, Editor. 2013: Philadelphia. p. 464-472.
18. Campochiaro, P.A., et al., *Intravitreal chemotactic and mitogenic activity. Implication of blood-retinal barrier breakdown.* Arch Ophthalmol, 1986. **104**(11): p. 1685-7.
19. Campochiaro, P.A., *Pathogenic mechanisms in proliferative vitreoretinopathy.* Arch Ophthalmol, 1997. **115**(2): p. 237-41.
20. Clarkson, J.G., W.R. Green, and D. Massof, *A histopathologic review of 168 cases of preretinal membrane.* Am J Ophthalmol, 1977. **84**(1): p. 1-17.
21. Machemer, R., D. van Horn, and T.M. Aaberg, *Pigment epithelial proliferation in human retinal detachment with massive periretinal proliferation.* Am J Ophthalmol, 1978. **85**(2): p. 181-91.
22. Kampik, A., et al., *Epiretinal and vitreous membranes. Comparative study of 56 cases.* Arch Ophthalmol, 1981. **99**(8): p. 1445-54.
23. Hiscott, P.S., I. Grierson, and D. McLeod, *Retinal pigment epithelial cells in epiretinal membranes: an immunohistochemical study.* Br J Ophthalmol, 1984. **68**(10): p. 708-15.
24. Jerdan, J.A., et al., *Proliferative vitreoretinopathy membranes. An immunohistochemical study.* Ophthalmology, 1989. **96**(6): p. 801-10.

25. Morino, I., et al., *Variation in epiretinal membrane components with clinical duration of the proliferative tissue.* Br J Ophthalmol, 1990. **74**(7): p. 393-9.
26. Wilkes, S.R., A.M. Mansour, and W.R. Green, *Proliferative vitreoretinopathy. Histopathology of retroretinal membranes.* Retina, 1987. **7**(2): p. 94-101.
27. Trese, M.T., D.B. Chandler, and R. Machemer, *Subretinal strands: ultrastructural features.* Graefes Arch Clin Exp Ophthalmol, 1985. **223**(1): p. 35-40.
28. Schwartz, D., et al., *Proliferative vitreoretinopathy. Ultrastructural study of 20 retroretinal membranes removed by vitreous surgery.* Retina, 1988. **8**(4): p. 275-81.
29. Hiscott, P., et al., *Cellular components of subretinal membranes in proliferative vitreoretinopathy.* Eye (Lond), 1989. **3 (Pt 5)**: p. 606-10.
30. Rodrigues, M.M., D.A. Newsome, and R. Machemer, *Further characterization of epiretinal membranes in human massive periretinal proliferation.* Current Eye Research, 1981. **1**(6): p. 311-5.
31. Van Horn, D.L., et al., *Glial cell proliferation in human retinal detachment with massive periretinal proliferation.* Am J Ophthalmol, 1977. **84**(3): p. 383-93.
32. Hiscott, P.S., et al., *Retinal and epiretinal glia--an immunohistochemical study.* Br J Ophthalmol, 1984. **68**(10): p. 698-707.
33. Foos, R.Y., *Vitreoretinal juncture--simple epiretinal membranes.* Albrecht Von Graefes Archiv fur Klinische und Experimentelle Ophthalmologie, 1974. **189**(4): p. 231-50.
34. Machemer, R. and H. Laqua, *Pigment epithelium proliferation in retinal detachment (massive periretinal proliferation).* Am J Ophthalmol, 1975. **80**(1): p. 1-23.
35. Walshe, R., et al., *Proliferative retinal diseases: myofibroblasts cause chronic vitreoretinal traction.* Br J Ophthalmol, 1992. **76**(9): p. 550-2.
36. Hiscott, P.S., I. Grierson, and D. McLeod, *Natural history of fibrocellular epiretinal membranes: a quantitative, autoradiographic, and immunohistochemical study.* Br J Ophthalmol, 1985. **69**(11): p. 810-23.
37. Vinores, S.A., et al., *Simultaneous expression of keratin and glial fibrillary acidic protein by the same cells in epiretinal membranes.* Invest Ophthalmol Vis Sci, 1992. **33**(12): p. 3361-6.
38. Vinores, S.A., et al., *Ultrastructural and immunocytochemical changes in retinal pigment epithelium, retinal glia, and fibroblasts in vitreous culture.* Invest Ophthalmol Vis Sci, 1990. **31**(12): p. 2529-45.
39. Baudouin, C., et al., *Immunohistologic study of epiretinal membranes in proliferative vitreoretinopathy.* Am J Ophthalmol, 1990. **110**(6): p. 593-8.
40. Esser, P., K. Heimann, and P. Wiedemann, *Macrophages in proliferative vitreoretinopathy and proliferative diabetic retinopathy: differentiation of subpopulations.* Br J Ophthalmol, 1993. **77**(11): p. 731-3.
41. Charteris, D.G., et al., *Proliferative vitreoretinopathy. Lymphocytes in epiretinal membranes.* Ophthalmology, 1992. **99**(9): p. 1364-7.
42. Limb, G.A., et al., *Proliferative vitreoretinopathy: an examination of the involvement of lymphocytes, adhesion molecules and HLA-DR antigens.* Graefes Arch Clin Exp Ophthalmol, 1993. **231**(6): p. 331-6.
43. Charteris, D.G., et al., *Inflammatory cells in proliferative vitreoretinopathy subretinal membranes.* Ophthalmology, 1993. **100**(1): p. 43-6.
44. Pastor, J.C., et al., *Proliferative vitreoretinopathy: A new concept of disease pathogenesis and practical consequences.* Prog Retin Eye Res, 2015.
45. Sethi, C.S., et al., *Glial remodeling and neural plasticity in human retinal detachment with proliferative vitreoretinopathy.* Invest Ophthalmol Vis Sci, 2005. **46**(1): p. 329-42.
46. Charteris, D.G., et al., *Intraretinal and periretinal pathology in anterior proliferative vitreoretinopathy.* Graefes Arch Clin Exp Ophthalmol, 2007. **245**(1): p. 93-100.

47. Lewis, G.P., et al., *Animal models of retinal detachment and reattachment: identifying cellular events that may affect visual recovery.* Eye (Lond), 2002. **16**(4): p. 375-87.
48. Garweg, J.G., C. Tappeiner, and M. Halberstadt, *Pathophysiology of proliferative vitreoretinopathy in retinal detachment.* Surv Ophthalmol, 2013. **58**(4): p. 321-9.
49. Pastor, J.C., et al., *Proliferative vitreoretinopathy: A new concept of disease pathogenesis and practical consequences.* Prog Retin Eye Res, 2016. **51**: p. 125-55.
50. Scheiffarth, O.F., et al., *Proteins of the extracellular matrix in vitreoretinal membranes.* Graefes Arch Clin Exp Ophthalmol, 1988. **226**(4): p. 357-61.
51. Salzmann, J., et al., *Matrix metalloproteinases and their natural inhibitors in fibrovascular membranes of proliferative diabetic retinopathy.* Br J Ophthalmol, 2000. **84**(10): p. 1091-6.
52. Vidaurri-Leal, J.S. and B.M. Glaser, *Effect of fibrin on morphologic characteristics of retinal pigment epithelial cells.* Arch Ophthalmol, 1984. **102**(9): p. 1376-9.
53. Glaser, B.M., A. Cardin, and B. Biscoe, *Proliferative vitreoretinopathy. The mechanism of development of vitreoretinal traction.* Ophthalmology, 1987. **94**(4): p. 327-32.
54. Charteris, D.G., *Proliferative vitreoretinopathy: pathobiology, surgical management, and adjunctive treatment.* Br J Ophthalmol, 1995. **79**(10): p. 953-60.
55. El-Ghrably, I.A., et al., *Detection of cytokine mRNA production in infiltrating cells in proliferative vitreoretinopathy using reverse transcription polymerase chain reaction.* Br J Ophthalmol, 1999. **83**(11): p. 1296-9.
56. *Vitrectomy with silicone oil or perfluoropropane gas in eyes with severe proliferative vitreoretinopathy: results of a randomized clinical trial. Silicone Study Report 2.* Arch Ophthalmol, 1992. **110**(6): p. 780-92.
57. Rhen, T. and J.A. Cidlowski, *Antiinflammatory action of glucocorticoids--new mechanisms for old drugs.* N Engl J Med, 2005. **353**(16): p. 1711-23.
58. Barnes, P.J., *How corticosteroids control inflammation: Quintiles Prize Lecture 2005.* Br J Pharmacol, 2006. **148**(3): p. 245-54.
59. Brown, H.M., G. Storey, and W.H. George, *Beclomethasone dipropionate: a new steroid aerosol for the treatment of allergic asthma.* Br Med J, 1972. **1**(5800): p. 585-90.
60. Barnes, P.J. and I.M. Adcock, *How do corticosteroids work in asthma?* Ann Intern Med, 2003. **139**(5 Pt 1): p. 359-70.
61. Wu, B., et al., *3D structure of human FK506-binding protein 52: implications for the assembly of the glucocorticoid receptor/Hsp90/immunophilin heterocomplex.* Proc Natl Acad Sci U S A, 2004. **101**(22): p. 8348-53.
62. Leung, D.Y., et al., *Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta.* J Exp Med, 1997. **186**(9): p. 1567-74.
63. Dostert, A. and T. Heinzel, *Negative glucocorticoid receptor response elements and their role in glucocorticoid action.* Curr Pharm Des, 2004. **10**(23): p. 2807-16.
64. Mittelstadt, P.R. and J.D. Ashwell, *Inhibition of AP-1 by the glucocorticoid-inducible protein GILZ.* J Biol Chem, 2001. **276**(31): p. 29603-10.
65. Banerjee PJ, C.D., Wong D, in *Vitreous in Health and Disease*, S. J, Editor. 2014: New York. p. 523-536.
66. Braunschweiger, P.G. and L.M. Schiffer, *Antiproliferative effects of corticosteroids in C3H/HeJ Mammary tumors and implications for sequential combination chemotherapy.* Cancer Res, 1981. **41**(9 Pt 1): p. 3324-30.
67. Tano, Y., et al., *Inhibition of intraocular proliferations with intravitreal corticosteroids.* Am J Ophthalmol, 1980. **89**(1): p. 131-6.
68. Tano, Y., D. Chandler, and R. Machemer, *Treatment of intraocular proliferation with intravitreal injection of triamcinolone acetonide.* Am J Ophthalmol, 1980. **90**(6): p. 810-6.

69. Chandler, D.B., et al., *The effect of triamcinolone acetonide on a refined experimental model of proliferative vitreoretinopathy.* Am J Ophthalmol, 1985. **99**(6): p. 686-90.
70. Rubsamen, P.E. and S.W. Cousins, *Therapeutic effect of periocular corticosteroids in experimental proliferative vitreoretinopathy.* Retina, 1997. **17**(1): p. 44-50.
71. Kaczmarek, R., et al., *Antiproliferative effects of preservative-free triamcinolone acetonide on cultured human retinal pigment epithelial cells.* Med Sci Monit, 2009. **15**(8): p. BR227-31.
72. Kuo, H.K., et al., *The Effects of Ozurdex(R) (Dexamethasone Intravitreal Implant) on Experimental Proliferative Vitreoretinopathy.* Ophthalmologica, 2015. **233**(3-4): p. 198-203.
73. Koerner, F., et al., *Postoperative retinal fibrosis--a controlled clinical study of systemic steroid therapy.* Graefes Arch Clin Exp Ophthalmol, 1982. **219**(6): p. 268-71.
74. Bali, E., et al., *The effect of a preoperative subconjuntival injection of dexamethasone on blood-retinal barrier breakdown following scleral buckling retinal detachment surgery: a prospective randomized placebo-controlled double blind clinical trial.* Graefes Arch Clin Exp Ophthalmol, 2010. **248**(7): p. 957-62.
75. Jonas, J.B., J.K. Hayler, and S. Panda-Jonas, *Intravitreal injection of crystalline cortisone as adjunctive treatment of proliferative vitreoretinopathy.* Br J Ophthalmol, 2000. **84**(9): p. 1064-7.
76. Sakamoto, T., et al., *Triamcinolone-assisted pars plana vitrectomy improves the surgical procedures and decreases the postoperative blood-ocular barrier breakdown.* Graefes Arch Clin Exp Ophthalmol, 2002. **240**(6): p. 423-9.
77. Munir, W.M., et al., *Intravitreal triamcinolone for treatment of complicated proliferative diabetic retinopathy and proliferative vitreoretinopathy.* Can J Ophthalmol, 2005. **40**(5): p. 598-604.
78. Cheema, R.A., et al., *Triamcinolone acetonide as an adjuvant in the surgical treatment of retinal detachment with proliferative vitreoretinopathy.* Ophthalmic Surg Lasers Imaging, 2007. **38**(5): p. 365-70.
79. Acar, N., et al., *Pars plana vitrectomy with and without triamcinolone acetonide assistance in pseudophakic retinal detachment complicated with proliferative vitreoretinopathy.* Japanese Journal of Ophthalmology, 2010. **54**(4): p. 331-7.
80. Chen, W., et al., *Midterm results of low-dose intravitreal triamcinolone as adjunctive treatment for proliferative vitreoretinopathy.* Retina, 2011. **31**(6): p. 1137-42.
81. Yamakiri, K., et al., *Reduced incidence of intraoperative complications in a multicenter controlled clinical trial of triamcinolone in vitrectomy.* Ophthalmology, 2007. **114**(2): p. 289-96.
82. Yamakiri, K., et al., *One-year results of a multicenter controlled clinical trial of triamcinolone in pars plana vitrectomy.* Graefes Arch Clin Exp Ophthalmol, 2008. **246**(7): p. 959-66.
83. Ahmadieh, H., et al., *Triamcinolone acetonide in silicone-filled eyes as adjunctive treatment for proliferative vitreoretinopathy: a randomized clinical trial.* Ophthalmology, 2008. **115**(11): p. 1938-43.
84. Koerner, F., U. Koerner-Stiefbold, and J.G. Garweg, *Systemic corticosteroids reduce the risk of cellophane membranes after retinal detachment surgery: a prospective randomized placebo-controlled double-blind clinical trial.* Graefes Arch Clin Exp Ophthalmol, 2012. **250**(7): p. 981-7.
85. Weijtens, O., et al., *Dexamethasone concentration in the subretinal fluid after a subconjunctival injection, a peribulbar injection, or an oral dose.* Ophthalmology, 2000. **107**(10): p. 1932-8.

86. Blumenkranz, M.S., A. Claflin, and A.S. Hajek, *Selection of therapeutic agents for intraocular proliferative disease. Cell culture evaluation.* Arch Ophthalmol, 1984. **102**(4): p. 598-604.
87. Cardillo, J.A., et al., *An intravitreal biodegradable sustained release naproxen and 5-fluorouracil system for the treatment of experimental post-traumatic proliferative vitreoretinopathy.* Br J Ophthalmol, 2004. **88**(9): p. 1201-5.
88. Sanders, D.R., et al., *Breakdown and reestablishment of blood-aqueous barrier with implant surgery.* Arch Ophthalmol, 1982. **100**(4): p. 588-90.
89. Sanders, D.R. and M. Kraff, *Steroidal and nonsteroidal anti-inflammatory agents. Effect on postsurgical inflammation and blood-aqueous humor barrier breakdown.* Arch Ophthalmol, 1984. **102**(10): p. 1453-6.
90. Blumenkranz, M.S., et al., *Fluorouracil for the treatment of massive periretinal proliferation.* Am J Ophthalmol, 1982. **94**(4): p. 458-67.
91. Stern, W.H., et al., *Fluorouracil therapy for proliferative vitreoretinopathy after vitrectomy.* Am J Ophthalmol, 1983. **96**(1): p. 33-42.
92. Rubsamen, P.E., et al., *Prevention of experimental proliferative vitreoretinopathy with a biodegradable intravitreal implant for the sustained release of fluorouracil.* Arch Ophthalmol, 1994. **112**(3): p. 407-13.
93. Berger, A.S., et al., *Intravitreal sustained release corticosteroid-5-fluoruracil conjugate in the treatment of experimental proliferative vitreoretinopathy.* Invest Ophthalmol Vis Sci, 1996. **37**(11): p. 2318-25.
94. Yang, C.S., et al., *An intravitreal sustained-release triamcinolone and 5-fluorouracil codrug in the treatment of experimental proliferative vitreoretinopathy.* Arch Ophthalmol, 1998. **116**(1): p. 69-77.
95. Blumenkranz, M., et al., *5-fluorouracil: new applications in complicated retinal detachment for an established antimetabolite.* Ophthalmology, 1984. **91**(2): p. 122-30.
96. Blankenship, G.W., *Evaluation of a single intravitreal injection of 5-fluorouracil in vitrectomy cases.* Graefes Arch Clin Exp Ophthalmol, 1989. **227**(6): p. 565-8.
97. Asaria, R.H., et al., *Adjuvant 5-fluorouracil and heparin prevents proliferative vitreoretinopathy : Results from a randomized, double-blind, controlled clinical trial.* Ophthalmology, 2001. **108**(7): p. 1179-83.
98. Charteris, D.G., et al., *A randomized controlled trial of combined 5-fluorouracil and low-molecular-weight heparin in management of established proliferative vitreoretinopathy.* Ophthalmology, 2004. **111**(12): p. 2240-5.
99. Kirmani, M., et al., *Antiproliferative drugs in the treatment of experimental proliferative vitreoretinopathy.* Retina, 1983. **3**(4): p. 269-72.
100. Santana, M., et al., *Daunomycin in the treatment of experimental proliferative vitreoretinopathy: retinal toxicity of intravitreal daunomycin in the rabbit.* Graefes Arch Clin Exp Ophthalmol, 1984. **221**(5): p. 210-3.
101. Verdoorn, C., et al., *Cellular migration, proliferation, and contraction. An in vitro approach to a clinical problem--proliferative vitreoretinopathy.* Arch Ophthalmol, 1986. **104**(8): p. 1216-9.
102. Weller, M., K. Heimann, and P. Wiedemann, *Cytotoxic effects of daunomycin on retinal pigment epithelium in vitro.* Graefes Arch Clin Exp Ophthalmol, 1987. **225**(3): p. 235-8.
103. Khawly, J.A., et al., *Daunorubicin treatment in a refined experimental model of proliferative vitreoretinopathy.* Graefes Arch Clin Exp Ophthalmol, 1991. **229**(5): p. 464-7.
104. Kumar, A., et al., *Experimental inhibition of proliferative vitreoretinopathy in retinal detachment using daunorubicin.* Indian Journal of Ophthalmology, 1994. **42**(1): p. 31-5.

105. Wiedemann, P., et al., *Daunomycin in the treatment of experimental proliferative vitreoretinopathy. Effective doses in vitro and in vivo.* Invest Ophthalmol Vis Sci, 1985. **26**(5): p. 719-25.
106. Esser, P., et al., *Intravitreal daunomycin induces multidrug resistance in proliferative vitreoretinopathy.* Invest Ophthalmol Vis Sci, 1998. **39**(1): p. 164-70.
107. Kuo, H.K., et al., *Attenuated glial reaction in experimental proliferative vitreoretinopathy treated with liposomal doxorubicin.* Invest Ophthalmol Vis Sci, 2012. **53**(6): p. 3167-74.
108. Wiedemann, P., et al., *Intraocular daunorubicin for the treatment and prophylaxis of traumatic proliferative vitreoretinopathy.* Am J Ophthalmol, 1987. **104**(1): p. 10-4.
109. Wiedemann, P., et al., *Daunomycin and silicone oil for the treatment of proliferative vitreoretinopathy.* Graefes Arch Clin Exp Ophthalmol, 1991. **229**(2): p. 150-2.
110. Wiedemann, P., et al., *Adjunctive daunorubicin in the treatment of proliferative vitreoretinopathy: results of a multicenter clinical trial. Daunomycin Study Group.* Am J Ophthalmol, 1998. **126**(4): p. 550-9.
111. Raczynska, K., et al., *[Vitrectomy with daunorubicin].* Klinika Oczna, 2004. **106**(3 Suppl): p. 481-4.
112. Campochiaro, P.A., S.F. Hackett, and B.P. Conway, *Retinoic acid promotes density-dependent growth arrest in human retinal pigment epithelial cells.* Invest Ophthalmol Vis Sci, 1991. **32**(1): p. 65-72.
113. Verstraeten, T., et al., *Effects of vitamin A on retinal pigment epithelial cells in vitro.* Invest Ophthalmol Vis Sci, 1992. **33**(10): p. 2830-8.
114. Giordano, G.G., M.F. Refojo, and M.H. Arroyo, *Sustained delivery of retinoic acid from microspheres of biodegradable polymer in PVR.* Invest Ophthalmol Vis Sci, 1993. **34**(9): p. 2743-51.
115. Yan, X.R., et al., *[Antiproliferative effect of sustained drug delivery system of all-trans retinoic acid implant into rabbit's vitreous cavity for treatment of proliferative vitreoretinopathy].* Zhonghua Yan Ke Za Zhi, 2003. **39**(10): p. 621-5.
116. Dong, X., et al., *Prevention of experimental proliferative vitreoretinopathy with a biodegradable intravitreal drug delivery system of all-trans retinoic acid.* Retina, 2006. **26**(2): p. 210-3.
117. Nakagawa, M., et al., *Retinoic acid in silicone and silicone-fluorosilicone copolymer oils in a rabbit model of proliferative vitreoretinopathy.* Invest Ophthalmol Vis Sci, 1995. **36**(12): p. 2388-95.
118. Veloso, A.A., Jr., et al., *13-cis-retinoic acid in silicone-fluorosilicone copolymer oil in a rabbit model of proliferative vitreoretinopathy.* Experimental Eye Research, 1997. **65**(3): p. 425-34.
119. Wu, W.C., et al., *Effects of retinoic acid on retinal pigment epithelium from excised membranes from proliferative vitreoretinopathy.* Journal of Ocular Pharmacology and Therapeutics, 2005. **21**(1): p. 44-54.
120. Chang, Y.C., et al., *All-trans retinoic acid remodels extracellular matrix and suppresses laminin-enhanced contractility of cultured human retinal pigment epithelial cells.* Experimental Eye Research, 2009. **88**(5): p. 900-9.
121. Fekrat, S., E. de Juan, Jr., and P.A. Campochiaro, *The effect of oral 13-cis-retinoic acid on retinal redetachment after surgical repair in eyes with proliferative vitreoretinopathy.* Ophthalmology, 1995. **102**(3): p. 412-8.
122. Chang, Y.C., D.N. Hu, and W.C. Wu, *Effect of oral 13-cis-retinoic acid treatment on postoperative clinical outcome of eyes with proliferative vitreoretinopathy.* Am J Ophthalmol, 2008. **146**(3): p. 440-446.

123. Weller, M., K. Heimann, and P. Wiedemann, *[Immunohistochemical findings in proliferative retinal diseases--on the significance of fibronectin, macrophages and transferrin].* Fortschritte der Ophthalmologie, 1989. **86**(2): p. 121-3.
124. Baudouin, C., et al., *Transferrin receptor expression by retinal pigment epithelial cells in proliferative vitreoretinopathy.* Invest Ophthalmol Vis Sci, 1992. **33**(10): p. 2822-9.
125. Davis, A.A., et al., *Selective inhibition of growing pigment epithelial cells by a receptor-directed immunotoxin.* Invest Ophthalmol Vis Sci, 1990. **31**(12): p. 2514-9.
126. Jaffe, G.J., et al., *Antitransferrin receptor immunotoxin inhibits proliferating human retinal pigment epithelial cells.* Arch Ophthalmol, 1990. **108**(8): p. 1163-8.
127. Handa, J.T., L.L. Houston, and G.J. Jaffe, *The antiproliferative effect of a transferrin-toxin on human retinal pigment epithelial cells and rabbit fibroblasts.* Invest Ophthalmol Vis Sci, 1993. **34**(12): p. 3419-28.
128. Hermsen, V.M., et al., *Long-term inhibition of cellular proliferation by immunotoxins.* Arch Ophthalmol, 1990. **108**(7): p. 1009-11.
129. Handa, J.T., M.A. Keithahn, and G.J. Jaffe, *Transferrin-ricin A chain toxin limits the development of experimental proliferative vitreoretinopathy.* Experimental Eye Research, 1996. **62**(6): p. 689-96.
130. Hoffmann, S., et al., *Selective killing of RPE with a vascular endothelial growth factor chimeric toxin.* Invest Ophthalmol Vis Sci, 2000. **41**(8): p. 2389-93.
131. van Bockxmeer, F.M., C.E. Martin, and I.J. Constable, *Models for assessing scar tissue inhibitors.* Retina, 1985. **5**(1): p. 47-60.
132. Campochiaro, P.A. and B.M. Glaser, *Mechanisms involved in retinal pigment epithelial cell chemotaxis.* Arch Ophthalmol, 1986. **104**(2): p. 277-80.
133. Lemor, M., S. de Bustros, and B.M. Glaser, *Low-dose colchicine inhibits astrocyte, fibroblast, and retinal pigment epithelial cell migration and proliferation.* Arch Ophthalmol, 1986. **104**(8): p. 1223-5.
134. Lemor, M., J.H. Yeo, and B.M. Glaser, *Oral colchicine for the treatment of experimental traction retinal detachment.* Arch Ophthalmol, 1986. **104**(8): p. 1226-9.
135. Raymond, M.C. and J.T. Thompson, *RPE-mediated collagen gel contraction. Inhibition by colchicine and stimulation by TGF-beta.* Invest Ophthalmol Vis Sci, 1990. **31**(6): p. 1079-86.
136. Pastor, J.C., et al., *Combined pharmacologic therapy in a rabbit model of proliferative vitreoretinopathy (PVR).* Ophthalmic Research, 2000. **32**(1): p. 25-9.
137. Cai, J., et al., *Cytotoxic effects of antiproliferative agents on human retinal glial cells in vitro.* International Ophthalmology, 2001. **24**(4): p. 225-31.
138. Berman, D.H. and G.M. Gombos, *Proliferative vitreoretinopathy: does oral low-dose colchicine have an inhibitory effect? A controlled study in humans.* Ophthalmic Surg, 1989. **20**(4): p. 268-72.
139. Yoo, J.S., et al., *cis-Hydroxyproline inhibits proliferation, collagen synthesis, attachment, and migration of cultured bovine retinal pigment epithelial cells.* Invest Ophthalmol Vis Sci, 1997. **38**(2): p. 520-8.
140. Yasukawa, T., et al., *Sustained release of cis-hydroxyproline in the treatment of experimental proliferative vitreoretinopathy in rabbits.* Graefes Arch Clin Exp Ophthalmol, 2002. **240**(8): p. 672-8.
141. Kon, C.H., et al., *A prospective study of matrix metalloproteinases in proliferative vitreoretinopathy.* Invest Ophthalmol Vis Sci, 1998. **39**(8): p. 1524-9.
142. Ozerdem, U., et al., *The effect of prinomastat (AG3340), a potent inhibitor of matrix metalloproteinases, on a subacute model of proliferative vitreoretinopathy.* Current Eye Research, 2000. **20**(6): p. 447-53.

143. Ozerdem, U., et al., *The effect of prinomastat (AG3340), a synthetic inhibitor of matrix metalloproteinases, on posttraumatic proliferative vitreoretinopathy.* Ophthalmic Research, 2001. **33**(1): p. 20-3.
144. El-Bradey, M.H., et al., *The effect of prinomastat (AG3340), a potent inhibitor of matrix metalloproteinase, on a new animal model of epiretinal membrane.* Retina, 2004. **24**(5): p. 783-9.
145. Blumenkranz, M.S.H., M.K, *Pharmacologic treatment of proliferative vitreoretinopathy: interrelationship with the coagulation cascade*, in *Medical and surgical retina, advances, controversies and management*, H.R. Lewis, S.J, Editor. 1993, Mosby: St Louis. p. 172.
146. Johnson, R.N. and G. Blankenship, *A prospective, randomized, clinical trial of heparin therapy for postoperative intraocular fibrin.* Ophthalmology, 1988. **95**(3): p. 312-7.
147. Williams, R.G., et al., *Does the presence of heparin and dexamethasone in the vitrectomy infusate reduce reproliferation in proliferative vitreoretinopathy?* Graefes Arch Clin Exp Ophthalmol, 1996. **234**(8): p. 496-503.
148. Iverson, D.A., et al., *Inhibition of intraocular fibrin formation following infusion of low-molecular-weight heparin during vitrectomy.* Arch Ophthalmol, 1991. **109**(3): p. 405-9.
149. Kon, C.H., et al., *Risk factors for proliferative vitreoretinopathy after primary vitrectomy: a prospective study.* Br J Ophthalmol, 2000. **84**(5): p. 506-11.
150. Collier, R., *Legumes, lemons and streptomycin: a short history of the clinical trial.* CMAJ, 2009. **180**(1): p. 23-4.
151. Bhatt, A., *Evolution of clinical research: a history before and beyond james lind.* Perspect Clin Res, 2010. **1**(1): p. 6-10.
152. Chalmers, I., et al., *The James Lind Library: explaining and illustrating the evolution of fair tests of medical treatments.* J R Coll Physicians Edinb, 2008. **38**(3): p. 259-64.
153. Flint, A., *A Contribution Toward the Natural History of Articular Rheumatism, Consisting of a Report of Thirteen Cases Treated Solely with Palliative Measures.* American Journal of Medical Science, 1863. **46**: p. 17-36.
154. *The Evolution of the Clinical Trials Process – A Brief History Lesson*. [cited 2015 14/07/2015]; Available from: http://www.psoriasiscouncil.org/docs/chapter_01.pdf
155. *STREPTOMYCIN treatment of pulmonary tuberculosis.* Br Med J, 1948. **2**(4582): p. 769-82.
156. Shuster, E., *Fifty years later: the significance of the Nuremberg Code.* N Engl J Med, 1997. **337**(20): p. 1436-40.
157. World Medical, A., *World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects.* JAMA, 2013. **310**(20): p. 2191-4.
158. McCully, S., *The UK Clinical Trials Regulations An introduction to the regulations andguidelines that govern clinical trials inthe UK*. 2011. p. 1-38.
159. Smithells, R.W. and C.G. Newman, *Recognition of thalidomide defects.* J Med Genet, 1992. **29**(10): p. 716-23.
160. Leck, I.M. and E.L. Millar, *Incidence of malformations since the introduction of thalidomide.* Br Med J, 1962. **2**(5296): p. 16-20.
161. Smithells, R.W., *Thalidomide and malformations in Liverpool.* Lancet, 1962. **1**(7242): p. 1270-3.
162. Smithells, R.W., *The incidence of limb and ear defects since the withdrawal of thalidomide.* Lancet, 1963. **1**(7290): p. 1095-7.
163. *THE PRINCIPLES OF ICH GCP*. 14/07/2015]; Available from: http://ichgcp.net/2-the-principles-of-ich-gcp-2.
164. *Health watchdogs and authorities*. Available from: www.nhs.uk/nhsengland/thenhs/healthregulators/pages/mhra.aspx

165. Esmaeli, B., et al., *Visual outcome and ocular survival after penetrating trauma. A clinicopathologic study.* Ophthalmology, 1995. **102**(3): p. 393-400.
166. McGwin, G., Jr., A. Xie, and C. Owsley, *Rate of eye injury in the United States.* Arch Ophthalmol, 2005. **123**(7): p. 970-6.
167. McGwin, G., Jr., et al., *Trends in eye injury in the United States, 1992-2001.* Invest Ophthalmol Vis Sci, 2006. **47**(2): p. 521-7.
168. Kuhn, F., et al., *Epidemiology of blinding trauma in the United States Eye Injury Registry.* Ophthalmic Epidemiol, 2006. **13**(3): p. 209-16.
169. Morris, D.S., et al., *The incidence of serious eye injury in Scotland: a prospective study.* Eye (Lond), 2014. **28**(1): p. 34-40.
170. *National Society to Prevent Blindness Vision Problems in the U.S. :a statistical analysis.* . 1980, New York: National Society to Prevent Blindness. p. 25-26.
171. Negrel, A.D. and B. Thylefors, *The global impact of eye injuries.* Ophthalmic Epidemiol, 1998. **5**(3): p. 143-69.
172. Desai, P., et al., *Incidence of cases of ocular trauma admitted to hospital and incidence of blinding outcome.* Br J Ophthalmol, 1996. **80**(7): p. 592-6.
173. Pinna, A., et al., *Epidemiology, visual outcome, and hospitalization costs of open globe injury in northern Sardinia, Italy.* Ophthalmic Epidemiol, 2007. **14**(5): p. 299-305.
174. Cillino, S., et al., *A five-year retrospective study of the epidemiological characteristics and visual outcomes of patients hospitalized for ocular trauma in a Mediterranean area.* BMC Ophthalmol, 2008. **8**: p. 6.
175. Kuhn, F., R. Morris, and C.D. Witherspoon, *Birmingham Eye Trauma Terminology (BETT): terminology and classification of mechanical eye injuries.* Ophthalmol Clin North Am, 2002. **15**(2): p. 139-43, v.
176. Stryjewski, T.P., C.M. Andreoli, and D. Eliott, *Retinal detachment after open globe injury.* Ophthalmology, 2014. **121**(1): p. 327-33.
177. Kuhn, F., et al., *The Ocular Trauma Score (OTS).* Ophthalmol Clin North Am, 2002. **15**(2): p. 163-5, vi.
178. Cardillo, J.A., et al., *Post-traumatic proliferative vitreoretinopathy. The epidemiologic profile, onset, risk factors, and visual outcome.* Ophthalmology, 1997. **104**(7): p. 1166-73.
179. Spiegel, D., et al., *Severe ocular trauma managed with primary pars plana vitrectomy and silicone oil.* Retina, 1997. **17**(4): p. 275-85.
180. Andreoli, M.T. and C.M. Andreoli, *Surgical rehabilitation of the open globe injury patient.* Am J Ophthalmol, 2012. **153**(5): p. 856-60.
181. Framme, C. and J. Roider, *[Epidemiology of open globe injuries].* Klin Monbl Augenheilkd, 1999. **215**(5): p. 287-93.
182. Mittra, R.A. and W.F. Mieler, *Controversies in the management of open-globe injuries involving the posterior segment.* Surv Ophthalmol, 1999. **44**(3): p. 215-25.
183. Sobaci, G., et al., *Deadly weapon-related open-globe injuries: outcome assessment by the ocular trauma classification system.* Am J Ophthalmol, 2000. **129**(1): p. 47-53.
184. Malbran, E., R. Dodds, and R. Hulsbus, *Traumatic retinal detachment.* Mod Probl Ophthalmol, 1972. **10**: p. 479-89.
185. Cox, M.S. and H.M. Freeman, *Retinal detachment due to ocular penetration. I. Clinical characteristics and surgical results.* Arch Ophthalmol, 1978. **96**(8): p. 1354-61.
186. Wong, T.Y. and J.M. Tielsch, *A population-based study on the incidence of severe ocular trauma in Singapore.* Am J Ophthalmol, 1999. **128**(3): p. 345-51.
187. Tano, Y., et al., *Glucocorticosteroid inhibition of intraocular proliferation after injury.* Am J Ophthalmol, 1981. **91**(2): p. 184-9.

188. Albini, T.A., et al., *Long-term retinal toxicity of intravitreal commercially available preserved triamcinolone acetonide (Kenalog) in rabbit eyes.* Invest Ophthalmol Vis Sci, 2007. **48**(1): p. 390-5.
189. Wickham, L., *Intensive anti-inflammatory therapy as an adjuvant treatment for established proliferative vitreoretinopathy*, in *ARVO*. 2007.
190. Diabetic Retinopathy Clinical Research, N., et al., *Three-year follow-up of a randomized trial comparing focal/grid photocoagulation and intravitreal triamcinolone for diabetic macular edema.* Arch Ophthalmol, 2009. **127**(3): p. 245-51.
191. Scott, I.U., et al., *A randomized trial comparing the efficacy and safety of intravitreal triamcinolone with standard care to treat vision loss associated with macular Edema secondary to branch retinal vein occlusion: the Standard Care vs Corticosteroid for Retinal Vein Occlusion (SCORE) study report 6.* Arch Ophthalmol, 2009. **127**(9): p. 1115-28.
192. Conway, M.D., et al., *Intravitreal triamcinolone acetonide for refractory chronic pseudophakic cystoid macular edema.* J Cataract Refract Surg, 2003. **29**(1): p. 27-33.
193. Challa, J.K., et al., *Exudative macular degeneration and intravitreal triamcinolone: 18 month follow up.* Aust N Z J Ophthalmol, 1998. **26**(4): p. 277-81.
194. Peyman, G.A., et al., *Triamcinolone acetonide as an aid to visualization of the vitreous and the posterior hyaloid during pars plana vitrectomy.* Retina, 2000. **20**(5): p. 554-5.
195. Vasconcelos-Santos, D.V., et al., *Secondary ocular hypertension after intravitreal injection of 4 mg of triamcinolone acetonide: incidence and risk factors.* Retina, 2008. **28**(4): p. 573-80.
196. Beer, P.M., et al., *Intraocular concentration and pharmacokinetics of triamcinolone acetonide after a single intravitreal injection.* Ophthalmology, 2003. **110**(4): p. 681-6.
197. Banerjee, P.J., et al., *A pilot study of intraocular use of intensive anti-inflammatory; triamcinolone acetonide to prevent proliferative vitreoretinopathy in eyes undergoing vitreoretinal surgery for open globe trauma; the adjuncts in ocular trauma (AOT) trial: study protocol for a randomised controlled trial.* Trials, 2013. **14**(1): p. 42.
198. Toerien, M., et al., *A review of reporting of participant recruitment and retention in RCTs in six major journals.* Trials, 2009. **10**: p. 52.
199. Sheng, I., et al., *A 10-year review of open-globe trauma in elderly patients at an urban hospital.* Retina, 2015. **35**(1): p. 105-10.
200. Jonas, J.B., *Intravitreal triamcinolone acetonide for diabetic retinopathy.* Dev Ophthalmol, 2007. **39**: p. 96-110.
201. Chin, H.S., et al., *Difference in clearance of intravitreal triamcinolone acetonide between vitrectomized and nonvitrectomized eyes.* Retina, 2005. **25**(5): p. 556-60.
202. Spitzer, M.S., et al., *The distribution, release kinetics, and biocompatibility of triamcinolone injected and dispersed in silicone oil.* Invest Ophthalmol Vis Sci, 2009. **50**(5): p. 2337-43.
203. Asaria, R.H., et al., *Silicone oil concentrates fibrogenic growth factors in the retro-oil fluid.* Br J Ophthalmol, 2004. **88**(11): p. 1439-42.
204. Marticorena, J., V. Romano, and F. Gomez-Ulla, *Sterile endophthalmitis after intravitreal injections.* Mediators Inflamm, 2012. **2012**: p. 928123.
205. Oliveira, R.C., et al., *Vitreous pharmacokinetics and retinal safety of intravitreal preserved versus non-preserved triamcinolone acetonide in rabbit eyes.* Curr Eye Res, 2012. **37**(1): p. 55-61.
206. Banerjee, P.J., et al., *Triamcinolone during pars plana vitrectomy for open globe trauma: a pilot randomised controlled clinical trial.* Br J Ophthalmol, 2015.
207. Peacock, J.L., et al., *Dichotomising continuous data while retaining statistical power using a distributional approach.* Stat Med, 2012. **31**(26): p. 3089-103.

208. Patel, N.N., et al., *Resources involved in managing retinal detachment complicated by proliferative vitreoretinopathy.* Retina, 2004. **24**(6): p. 883-7.
209. Binder, S., et al., *Radiation therapy in proliferative vitreoretinopathy. A prospective randomized study.* Graefes Arch Clin Exp Ophthalmol, 1994. **232**(4): p. 211-4.
210. London, N.J., A. Chiang, and J.A. Haller, *The dexamethasone drug delivery system: indications and evidence.* Adv Ther, 2011. **28**(5): p. 351-66.
211. Kwak, H.W. and D.J. D'Amico, *Evaluation of the retinal toxicity and pharmacokinetics of dexamethasone after intravitreal injection.* Arch Ophthalmol, 1992. **110**(2): p. 259-66.
212. Graham, R.O. and G.A. Peyman, *Intravitreal injection of dexamethasone. Treatment of experimentally induced endophthalmitis.* Arch Ophthalmol, 1974. **92**(2): p. 149-54.
213. Chang-Lin, J.E., et al., *Pharmacokinetics and pharmacodynamics of a sustained-release dexamethasone intravitreal implant.* Invest Ophthalmol Vis Sci, 2011. **52**(1): p. 80-6.
214. Migita, K., et al., *Apoptosis induction in human peripheral blood T lymphocytes by high-dose steroid therapy.* Transplantation, 1997. **63**(4): p. 583-7.
215. Leussink, V.I., et al., *High-dose methylprednisolone therapy in multiple sclerosis induces apoptosis in peripheral blood leukocytes.* Arch Neurol, 2001. **58**(1): p. 91-7.
216. Weijtens, O., et al., *Intraocular penetration and systemic absorption after topical application of dexamethasone disodium phosphate.* Ophthalmology, 2002. **109**(10): p. 1887-91.
217. Weijtens, O., et al., *Dexamethasone concentration in vitreous and serum after oral administration.* Am J Ophthalmol, 1998. **125**(5): p. 673-9.
218. Weijtens, O., et al., *Peribulbar corticosteroid injection: vitreal and serum concentrations after dexamethasone disodium phosphate injection.* Am J Ophthalmol, 1997. **123**(3): p. 358-63.
219. Weijtens, O., et al., *High concentration of dexamethasone in aqueous and vitreous after subconjunctival injection.* Am J Ophthalmol, 1999. **128**(2): p. 192-7.
220. Gan, I.M., et al., *Effect of intravitreal dexamethasone on vitreous vancomycin concentrations in patients with suspected postoperative bacterial endophthalmitis.* Graefes Arch Clin Exp Ophthalmol, 2005. **243**(11): p. 1186-9.
221. Haller, J.A., et al., *Randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with macular edema due to retinal vein occlusion.* Ophthalmology, 2010. **117**(6): p. 1134-1146 e3.
222. Lowder, C., et al., *Dexamethasone intravitreal implant for noninfectious intermediate or posterior uveitis.* Arch Ophthalmol, 2011. **129**(5): p. 545-53.
223. Boyer, D.S., et al., *Three-year, randomized, sham-controlled trial of dexamethasone intravitreal implant in patients with diabetic macular edema.* Ophthalmology, 2014. **121**(10): p. 1904-14.
224. Mehta, H., M. Gillies, and S. Fraser-Bell, *Perspective on the role of Ozurdex (dexamethasone intravitreal implant) in the management of diabetic macular oedema.* Ther Adv Chronic Dis, 2015. **6**(5): p. 234-45.
225. Lee, S.S., et al., *Vitreous VEGF clearance is increased after vitrectomy.* Invest Ophthalmol Vis Sci, 2010. **51**(4): p. 2135-8.
226. Schindler, R.H., et al., *The clearance of intravitreal triamcinolone acetonide.* Am J Ophthalmol, 1982. **93**(4): p. 415-7.
227. Jarus, G., et al., *Clearance of intravitreal fluorouracil. Normal and aphakic vitrectomized eyes.* Ophthalmology, 1985. **92**(1): p. 91-6.
228. Doft, B.H., et al., *Amphotericin clearance in vitrectomized versus nonvitrectomized eyes.* Ophthalmology, 1985. **92**(11): p. 1601-5.
229. Chang-Lin, J.E., et al., *Pharmacokinetics of a sustained-release dexamethasone intravitreal implant in vitrectomized and nonvitrectomized eyes.* Invest Ophthalmol Vis Sci, 2011. **52**(7): p. 4605-9.

230. Boyer, D.S., et al., *Dexamethasone intravitreal implant for treatment of diabetic macular edema in vitrectomized patients.* Retina, 2011. **31**(5): p. 915-23.
231. Adan, A., et al., *Dexamethasone intravitreal implant for treatment of uveitic persistent cystoid macular edema in vitrectomized patients.* Retina, 2013. **33**(7): p. 1435-40.
232. Reibaldi, M., et al., *Resolution of Persistent Cystoid Macular Edema due to Central Retinal Vein Occlusion in a Vitrectomized Eye following Intravitreal Implant of Dexamethasone 0.7 mg.* Case Rep Ophthalmol, 2012. **3**(1): p. 30-4.
233. Georgalas, I., et al., *Post-operative refractory cystoid macular edema in a vitrectomized eye treated with slow-release dexamethasone implant (Ozurdex).* Cutan Ocul Toxicol, 2015. **34**(3): p. 257-9.
234. Maltman, J., *Allergan data on file: Technical Report Number PD-TMEMO-00291*.
235. Banerjee, P.J., C. Bunce, and D.G. Charteris, *Ozurdex (a slow-release dexamethasone implant) in proliferative vitreoretinopathy: study protocol for a randomised controlled trial.* Trials, 2013. **14**: p. 358.
236. *Ozurdex Summary of Product Characteristics*. 2015; Available from: https://www.medicines.org.uk/emc/medicine/23422
237. Bansal, R., et al., *Wandering Ozurdex((R)) implant.* J Ophthalmic Inflamm Infect, 2011.
238. Pardo-Lopez, D., et al., *Anterior chamber migration of dexametasone intravitreal implant (Ozurdex(R)).* Graefes Arch Clin Exp Ophthalmol, 2011.
239. Nash, R., et al., *Ophthalmic Statistics Note 4: analysing data from randomised controlled trials with baseline and follow-up measurements.* Br J Ophthalmol, 2014. **98**(11): p. 1467-9.
240. Jabs, D.A., et al., *Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop.* Am J Ophthalmol, 2005. **140**(3): p. 509-16.
241. Banerjee, P.J., et al., *Neurotrophic corneal ulceration after retinal detachment surgery with retinectomy and endolaser: a case series.* JAMA Ophthalmol, 2014. **132**(6): p. 750-2.
242. Johnson, S.M., *Neurotrophic corneal defects after diode laser cycloablation.* Am J Ophthalmol, 1998. **126**(5): p. 725-7.
243. Patel, J.I., et al., *Dilated pupils and loss of accommodation following diode panretinal photocoagulation with sub-tenon local anaesthetic in four cases.* Eye (Lond), 2002. **16**(5): p. 628-32.
244. Menchini, U., et al., *Argon versus krypton panretinal photocoagulation side effects on the anterior segment.* Ophthalmologica, 1990. **201**(2): p. 66-70.
245. Afshar, A.R., et al., *Dexamethasone Intravitreal Implant Trapped at the Macula in a Silicone Oil-filled Eye.* Ophthalmology, 2013. **120**(12): p. 2748-2749 e1.
246. Banerjee, P.J., P. Petrou, and D.G. Charteris, *Re: Afshar et al.: Dexamethasone intravitreal implant trapped at the macula in a silicone oil-filled eye (Ophthalmology 2013;120:2748-9).* Ophthalmology, 2014. **121**(10): p. e55-7.
247. Meyer, C.H., et al., *Release and velocity of micronized dexamethasone implants with an intravitreal drug delivery system: kinematic analysis with a high-speed camera.* Retina, 2012. **32**(10): p. 2133-40.
248. Panjaphongse, R., et al., *Kinematic study of ozurdex injection in balanced salt solution: modeling the behavior of an injectable drug delivery device in vitrectomized eyes.* J Ocul Pharmacol Ther, 2015. **31**(3): p. 174-8.
249. Reimann, C. *Speed of Ozurdex Injection*. 2010 [cited 2015 15/07/2015]; Available from: http://eyetube.net/video/speed-of-ozurdex-injection/.
250. Banerjee, P.J., et al., *Spontaneous relocation of a trapped retrolenticular slow-release dexamethasone implant (Ozurdex) in a silicone oil-filled eye of a pseudophakic patient.* Eye (Lond), 2014. **28**(8): p. 1036-7.

251. *Vitrectomy with silicone oil or sulfur hexafluoride gas in eyes with severe proliferative vitreoretinopathy: results of a randomized clinical trial. Silicone Study Report 1.* Arch Ophthalmol, 1992. **110**(6): p. 770-9.
252. Benson, S.E., et al., *Analysis of the macula with optical coherence tomography after successful surgery for proliferative vitreoretinopathy.* Arch Ophthalmol, 2005. **123**(12): p. 1651-6.
253. Banker, T.P., et al., *Epiretinal membrane and cystoid macular edema after retinal detachment repair with small-gauge pars plana vitrectomy.* Eur J Ophthalmol, 2015: p. 0.
254. Scholl, S., J. Kirchhof, and A.J. Augustin, *Pathophysiology of macular edema.* Ophthalmologica, 2010. **224 Suppl 1**: p. 8-15.
255. Limb, G.A., et al., *Expression of mRNA coding for TNF alpha, IL-1 beta and IL-6 by cells infiltrating retinal membranes.* Graefes Arch Clin Exp Ophthalmol, 1994. **232**(11): p. 646-51.
256. Limb, G.A., et al., *Distribution of cytokine proteins within epiretinal membranes in proliferative vitreoretinopathy.* Curr Eye Res, 1994. **13**(11): p. 791-8.
257. Limb, G.A., et al., *Distribution of TNF alpha and its reactive vascular adhesion molecules in fibrovascular membranes of proliferative diabetic retinopathy.* Br J Ophthalmol, 1996. **80**(2): p. 168-73.
258. Limb, G.A., et al., *Evidence of chronic inflammation in retina excised after relaxing retinotomy for anterior proliferative vitreoretinopathy.* Graefes Arch Clin Exp Ophthalmol, 1996. **234**(4): p. 213-20.
259. Ichhpujani, P., A. Jindal, and L. Jay Katz, *Silicone oil induced glaucoma: a review.* Graefes Arch Clin Exp Ophthalmol, 2009. **247**(12): p. 1585-93.
260. Barr, C.C., et al., *Postoperative intraocular pressure abnormalities in the Silicone Study. Silicone Study Report 4.* Ophthalmology, 1993. **100**(11): p. 1629-35.
261. Henderer, J.D., et al., *Elevated intraocular pressure and hypotony following silicone oil retinal tamponade for complex retinal detachment: incidence and risk factors.* Arch Ophthalmol, 1999. **117**(2): p. 189-95.
262. Honavar, S.G., et al., *Glaucoma after pars plana vitrectomy and silicone oil injection for complicated retinal detachments.* Ophthalmology, 1999. **106**(1): p. 169-76; discussion 177.
263. Jonas, J.B., et al., *Intraocular pressure and silicone oil endotamponade.* J Glaucoma, 2001. **10**(2): p. 102-8.
264. Romano, M.R., et al., *Intraocular pressure changes following the use of silicone oil or Densiron 68 as endotamponade in pars plana vitrectomy.* Clin Ophthalmol, 2010. **4**: p. 1391-6.
265. Al-Jazzaf, A.M., P.A. Netland, and S. Charles, *Incidence and management of elevated intraocular pressure after silicone oil injection.* J Glaucoma, 2005. **14**(1): p. 40-6.
266. Wang, L., J. Liu, and T. Lu, *Clinical analysis of early and mid-late elevated intraocular pressure after silicone oil injection.* Eye Sci, 2014. **29**(2): p. 85-9.
267. Kersey, J.P. and D.C. Broadway, *Corticosteroid-induced glaucoma: a review of the literature.* Eye (Lond), 2006. **20**(4): p. 407-16.
268. McLean, J., *Use of ACTH and Cortisone.* Trans Am Ophthalmol Soc 1950. **48**: p. 293–296.
269. Francois, J., *Cortisone et tension oculaire*Ann D'Oculist, 1954. **187**: p. 805.
270. Smith, C.L., *"Corticosteroid glaucoma" a summary and review of the literaure.* Am J Med Sci, 1966. **252**(2): p. 239-44.
271. Armaly, M.F., *Effect of Corticosteroids on Intraocular Pressure and Fluid Dynamics. Ii. The Effect of Dexamethasone in the Glaucomatous Eye.* Arch Ophthalmol, 1963. **70**: p. 492-9.

272. Armaly, M.F., *Effect of Corticosteroids on Intraocular Pressure and Fluid Dynamics. I. The Effect of Dexamethasone in the Normal Eye.* Arch Ophthalmol, 1963. **70**: p. 482-91.
273. Francois, J., *Corticosteroid glaucoma.* Ann Ophthalmol, 1977. **9**(9): p. 1075-80.
274. Wordinger, R.J. and A.F. Clark, *Effects of glucocorticoids on the trabecular meshwork: towards a better understanding of glaucoma.* Prog Retin Eye Res, 1999. **18**(5): p. 629-67.
275. Huang, J., *Surgical Management of Complications due to Uveitis*, in *Ocular Inflammatory Disease and Uveitis Manual DIAGNOSIS AND TREATMENT*, P. Gaudio, Editor. 2010, Wolters Klewer: Philadelphia. p. 238.
276. Kim, S.W., et al., *Risk factors for the development of transient hypotony after silicone oil removal.* Retina, 2010. **30**(8): p. 1228-36.
277. Denniston, A.K.M.P., *Anatomy and Physiology Neural Retina*, in *Oxford Handbook of Ophthalmology*. 2009, Oxford University Press. p. 456.
278. Huang, D., et al., *Optical coherence tomography.* Science, 1991. **254**(5035): p. 1178-81.
279. Swanson, E.A., et al., *In vivo retinal imaging by optical coherence tomography.* Opt Lett, 1993. **18**(21): p. 1864-6.
280. Hee, M.R., et al., *Optical coherence tomography of macular holes.* Ophthalmology, 1995. **102**(5): p. 748-56.
281. Toth, C.A., et al., *A comparison of retinal morphology viewed by optical coherence tomography and by light microscopy.* Arch Ophthalmol, 1997. **115**(11): p. 1425-8.
282. Huang, Y., et al., *Relation of optical coherence tomography to microanatomy in normal and rd chickens.* Invest Ophthalmol Vis Sci, 1998. **39**(12): p. 2405-16.
283. Drexler, W., et al., *Ultrahigh-resolution ophthalmic optical coherence tomography.* Nat Med, 2001. **7**(4): p. 502-7.
284. Zawadzki, R.J., et al., *Adaptive-optics optical coherence tomography for high-resolution and high-speed 3D retinal in vivo imaging.* Opt Express, 2005. **13**(21): p. 8532-8546.
285. Spaide, R.F. and C.A. Curcio, *Anatomical correlates to the bands seen in the outer retina by optical coherence tomography: literature review and model.* Retina, 2011. **31**(8): p. 1609-19.
286. Curcio, C.A., et al., *Human chorioretinal layer thicknesses measured in macula-wide, high-resolution histologic sections.* Invest Ophthalmol Vis Sci, 2011. **52**(7): p. 3943-54.
287. Staurenghi, G., et al., *Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the IN*OCT consensus.* Ophthalmology, 2014. **121**(8): p. 1572-8.
288. Machemer, R., *Experimental retinal detachment in the owl monkey. II. Histology of retina and pigment epithelium.* Am J Ophthalmol, 1968. **66**(3): p. 396-410.
289. Nork, T.M., et al., *Selective loss of blue cones and rods in human retinal detachment.* Arch Ophthalmol, 1995. **113**(8): p. 1066-73.
290. Cook, B., et al., *Apoptotic photoreceptor degeneration in experimental retinal detachment.* Invest Ophthalmol Vis Sci, 1995. **36**(6): p. 990-6.
291. Chang, C.J., et al., *Apoptotic photoreceptor cell death after traumatic retinal detachment in humans.* Arch Ophthalmol, 1995. **113**(7): p. 880-6.
292. Arroyo, J.G., et al., *Photoreceptor apoptosis in human retinal detachment.* Am J Ophthalmol, 2005. **139**(4): p. 605-10.
293. Fisher, S.K. and G.P. Lewis, *Muller cell and neuronal remodeling in retinal detachment and reattachment and their potential consequences for visual recovery: a review and reconsideration of recent data.* Vision Res, 2003. **43**(8): p. 887-97.
294. Hisatomi, T., et al., *Critical role of photoreceptor apoptosis in functional damage after retinal detachment.* Curr Eye Res, 2002. **24**(3): p. 161-72.
295. Anderson, D.H., et al., *Morphological recovery in the reattached retina.* Invest Ophthalmol Vis Sci, 1986. **27**(2): p. 168-83.

296. Guerin, C.J., et al., *Retinal reattachment of the primate macula. Photoreceptor recovery after short-term detachment.* Invest Ophthalmol Vis Sci, 1989. **30**(8): p. 1708-25.
297. Guerin, C.J., et al., *Recovery of photoreceptor outer segment length and analysis of membrane assembly rates in regenerating primate photoreceptor outer segments.* Invest Ophthalmol Vis Sci, 1993. **34**(1): p. 175-83.
298. Sakai, T., et al., *Cone photoreceptor recovery after experimental detachment and reattachment: an immunocytochemical, morphological, and electrophysiological study.* Invest Ophthalmol Vis Sci, 2003. **44**(1): p. 416-25.
299. Kroll, A.J. and R. Machemer, *Experimental retinal detachment in the owl monkey. V. Electron microscopy of the reattached retina.* Am J Ophthalmol, 1969. **67**(1): p. 117-30.
300. Wolfensberger, T.J. and M. Gonvers, *Optical coherence tomography in the evaluation of incomplete visual acuity recovery after macula-off retinal detachments.* Graefes Arch Clin Exp Ophthalmol, 2002. **240**(2): p. 85-9.
301. Benson, S.E., et al., *Optical coherence tomography analysis of the macula after vitrectomy surgery for retinal detachment.* Ophthalmology, 2006. **113**(7): p. 1179-83.
302. Ricker, L.J., et al., *Persistent subfoveal fluid and increased preoperative foveal thickness impair visual outcome after macula-off retinal detachment repair.* Retina, 2011. **31**(8): p. 1505-12.
303. Seo, J.H., et al., *Influence of persistent submacular fluid on visual outcome after successful scleral buckle surgery for macula-off retinal detachment.* Am J Ophthalmol, 2008. **145**(5): p. 915-22.
304. Smith, A.J., et al., *High-resolution Fourier-domain optical coherence tomography and microperimetric findings after macula-off retinal detachment repair.* Ophthalmology, 2008. **115**(11): p. 1923-9.
305. Delolme, M.P., et al., *Anatomical and functional macular changes after rhegmatogenous retinal detachment with macula off.* Am J Ophthalmol, 2012. **153**(1): p. 128-36.
306. Schocket, L.S., et al., *Ultrahigh-resolution optical coherence tomography in patients with decreased visual acuity after retinal detachment repair.* Ophthalmology, 2006. **113**(4): p. 666-72.
307. Lai, W.W., et al., *Simultaneous spectral domain OCT and fundus autofluorescence imaging of the macula and microperimetric correspondence after successful repair of rhegmatogenous retinal detachment.* Br J Ophthalmol, 2010. **94**(3): p. 311-8.
308. Gharbiya, M., et al., *Correlation between spectral-domain optical coherence tomography findings and visual outcome after primary rhegmatogenous retinal detachment repair.* Retina, 2012. **32**(1): p. 43-53.
309. Sheth, S., et al., *Spectral domain-optical coherence tomography study of retinas with a normal foveal contour and thickness after retinal detachment surgery.* Retina, 2010. **30**(5): p. 724-32.
310. Rashid, S., et al., *Five-year follow-up of macular morphologic changes after rhegmatogenous retinal detachment repair: Fourier domain OCT findings.* Retina, 2013. **33**(10): p. 2049-58.
311. Wakabayashi, T., et al., *Foveal microstructure and visual acuity after retinal detachment repair: imaging analysis by Fourier-domain optical coherence tomography.* Ophthalmology, 2009. **116**(3): p. 519-28.
312. Kim, J.H., et al., *Topographic changes of retinal layers after resolution of acute retinal detachment.* Invest Ophthalmol Vis Sci, 2012. **53**(11): p. 7316-21.
313. Hagimura, N., et al., *Optical coherence tomography of the neurosensory retina in rhegmatogenous retinal detachment.* Am J Ophthalmol, 2000. **129**(2): p. 186-90.

314. Nakanishi, H., et al., *Spectral-domain optical coherence tomography imaging of the detached macula in rhegmatogenous retinal detachment.* Retina, 2009. **29**(2): p. 232-42.
315. Maruko, I., et al., *Morphologic changes in the outer layer of the detached retina in rhegmatogenous retinal detachment and central serous chorioretinopathy.* Am J Ophthalmol, 2009. **147**(3): p. 489-494 e1.
316. Abouzeid, H. and T.J. Wolfensberger, *Macular recovery after retinal detachment.* Acta Ophthalmol Scand, 2006. **84**(5): p. 597-605.
317. Mowatt, L., et al., *Correlation of visual recovery with macular height in macula-off retinal detachments.* Eye (Lond), 2010. **24**(2): p. 323-7.
318. Hassan, T.S., et al., *The effect of duration of macular detachment on results after the scleral buckle repair of primary, macula-off retinal detachments.* Ophthalmology, 2002. **109**(1): p. 146-52.
319. Shimoda, Y., et al., *Restoration of photoreceptor outer segment after vitrectomy for retinal detachment.* Am J Ophthalmol, 2010. **149**(2): p. 284-90.
320. Rii, T., et al., *Foveal cone outer segment tips line and disruption artifacts in spectral-domain optical coherence tomographic images of normal eyes.* Am J Ophthalmol, 2012. **153**(3): p. 524-529 e1.
321. Bunt-Milam, A.H., et al., *Zonulae adherentes pore size in the external limiting membrane of the rabbit retina.* Invest Ophthalmol Vis Sci, 1985. **26**(10): p. 1377-80.
322. Spitznas, M., *[The fine structure of the so-called outer limiting membrane in the human retina].* Albrecht Von Graefes Arch Klin Exp Ophthalmol, 1970. **180**(1): p. 44-56.
323. Lewis, G.P. and S.K. Fisher, *Up-regulation of glial fibrillary acidic protein in response to retinal injury: its potential role in glial remodeling and a comparison to vimentin expression.* Int Rev Cytol, 2003. **230**: p. 263-90.
324. Wickham, L. and D.G. Charteris, *Glial cell changes of the human retina in proliferative vitreoretinopathy.* Dev Ophthalmol, 2009. **44**: p. 37-45.
325. Hogan, M.J., S.J. Kimura, and P. Thygeson, *Signs and symptoms of uveitis. I. Anterior uveitis.* Am J Ophthalmol, 1959. **47**(5 Pt 2): p. 155-70.
326. Nussenblatt, R.B., et al., *Standardization of vitreal inflammatory activity in intermediate and posterior uveitis.* Ophthalmology, 1985. **92**(4): p. 467-71.
327. Kempen, J.H., et al., *Interobserver agreement in grading activity and site of inflammation in eyes of patients with uveitis.* Am J Ophthalmol, 2008. **146**(6): p. 813-8 e1.
328. Lin, P., E.B. Suhler, and J.T. Rosenbaum, *The future of uveitis treatment.* Ophthalmology, 2014. **121**(1): p. 365-76.
329. Denniston, A.K. and A.D. Dick, *Systemic therapies for inflammatory eye disease: past, present and future.* BMC Ophthalmol, 2013. **13**: p. 18.
330. Ladas, J.G., et al., *Laser flare-cell photometry: methodology and clinical applications.* Surv Ophthalmol, 2005. **50**(1): p. 27-47.
331. Sawa, M., et al., *New quantitative method to determine protein concentration and cell number in aqueous in vivo.* Jpn J Ophthalmol, 1988. **32**(2): p. 132-42.
332. Sawa, M., *Clinical application of laser flare-cell meter.* Jpn J Ophthalmol, 1990. **34**(3): p. 346-63.
333. de Ancos, E., N. Pittet, and C.P. Herbort, *[Quantitative measurement of inflammation in HLA-B27 acute anterior uveitis using the Kowa FC-100 laser flare-cell meter].* Klin Monbl Augenheilkd, 1994. **204**(5): p. 330-3.
334. Guex-Crosier, Y., N. Pittet, and C.P. Herbort, *Sensitivity of laser flare photometry to monitor inflammation in uveitis of the posterior segment.* Ophthalmology, 1995. **102**(4): p. 613-21.

335. Guex-Crosier, Y., N. Pittet, and C.P. Herbort, *Evaluation of laser flare-cell photometry in the appraisal and management of intraocular inflammation in uveitis.* Ophthalmology, 1994. **101**(4): p. 728-35.
336. Dick, H.B., et al., *Inflammation after sclerocorneal versus clear corneal tunnel phacoemulsification.* Ophthalmology, 2000. **107**(2): p. 241-7.
337. Miyake, K., et al., *Comparison of diclofenac and fluorometholone in preventing cystoid macular edema after small incision cataract surgery: a multicentered prospective trial.* Jpn J Ophthalmol, 2000. **44**(1): p. 58-67.
338. Keane, P.A., et al., *Objective measurement of vitreous inflammation using optical coherence tomography.* Ophthalmology, 2014. **121**(9): p. 1706-14.
339. Keane, P.A., et al., *Automated Analysis of Vitreous Inflammation Using Spectral-Domain Optical Coherence Tomography.* Transl Vis Sci Technol, 2015. **4**(5): p. 4.
340. Zarranz-Ventura, J., et al., *Evaluation of Objective Vitritis Grading Method Using Optical Coherence Tomography: Influence of Phakic Status and Previous Vitrectomy.* Am J Ophthalmol, 2015.
341. Forrester J, D.A., McMenamin P, Lee W, *Anatomy of the Eye and Orbit*, in *The Eye; Basic Science in Practice*. 2002, Saunders: Toronto. p. 47.
342. Bringmann, A., et al., *Muller cells in the healthy and diseased retina.* Progress in Retinal and Eye Research, 2006. **25**(4): p. 397-424.
343. Newman, E. and A. Reichenbach, *The Muller cell: a functional element of the retina.* Trends in Neurosciences, 1996. **19**(8): p. 307-12.
344. Pellegrini, G., et al., *Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium.* Lancet, 1997. **349**(9057): p. 990-3.
345. Raymond, P.A. and P.F. Hitchcock, *Retinal regeneration: common principles but a diversity of mechanisms.* Adv.Neurol., 1997. **72**: p. 171-184.
346. Raymond, P.A., et al., *Molecular characterization of retinal stem cells and their niches in adult zebrafish.* BMC Dev Biol, 2006. **6**: p. 36.
347. Fischer, A.J. and T.A. Reh, *Muller glia are a potential source of neural regeneration in the postnatal chicken retina.* Nat Neurosci, 2001. **4**(3): p. 247-52.
348. Ooto, S., et al., *Potential for neural regeneration after neurotoxic injury in the adult mammalian retina.* Proc.Natl.Acad.Sci.U.S.A, 2004. **101**(37): p. 13654-13659.
349. Das, A.V., et al., *Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling.* Developmental Biology, 2006. **299**(1): p. 283-302.
350. Limb, G.A., et al., *In vitro characterization of a spontaneously immortalized human Muller cell line (MIO-M1).* Invest Ophthalmol Vis Sci, 2002. **43**(3): p. 864-9.
351. Lawrence, J.M., et al., *MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics.* Stem Cells, 2007. **25**(8): p. 2033-43.
352. Bhatia, B., et al., *Distribution of Muller stem cells within the neural retina: evidence for the existence of a ciliary margin-like zone in the adult human eye.* Experimental Eye Research, 2009. **89**(3): p. 373-82.
353. Singhal, S., et al., *Human Muller glia with stem cell characteristics differentiate into retinal ganglion cell (RGC) precursors in vitro and partially restore RGC function in vivo following transplantation.* Stem Cells Transl Med, 2012. **1**(3): p. 188-99.
354. Giannelli, S.G., et al., *Adult human Muller glia cells are a highly efficient source of rod photoreceptors.* Stem Cells, 2011. **29**(2): p. 344-56.
355. Bringmann, A., et al., *Cellular signaling and factors involved in Muller cell gliosis: neuroprotective and detrimental effects.* Prog Retin Eye Res, 2009. **28**(6): p. 423-51.
356. Tezel, G., *The role of glia, mitochondria, and the immune system in glaucoma.* Investigative ophthalmology & visual science, 2009. **50**(3): p. 1001-12.

357. Tezel, G., *TNF-alpha signaling in glaucomatous neurodegeneration.* Prog Brain Res, 2008. **173**: p. 409-21.
358. Giulian, D., et al., *The impact of microglia-derived cytokines upon gliosis in the CNS.* Dev Neurosci, 1994. **16**(3-4): p. 128-36.
359. Limb, G.A., et al., *Soluble TNF receptors in vitreoretinal proliferative disease.* Invest Ophthalmol Vis Sci, 2001. **42**(7): p. 1586-91.
360. Close, J.L., B. Gumuscu, and T.A. Reh, *Retinal neurons regulate proliferation of postnatal progenitors and Muller glia in the rat retina via TGF beta signaling.* Development, 2005. **132**(13): p. 3015-26.

8 Appendices

8.1 Appendix 1: Adjuncts in Ocular Trauma Trial Case Report Form Pack

Moorfields Eye Hospital NHS
NHS Foundation Trust

Completing Case Report Forms (CRFs)

This document has been created to provide guidelines about completing clinical trial case report forms at Moorfields Eye Hospital (MEH). The information has been extracted from the revised MEH SOP documents that are being developed by the Research & Development department at Moorfields.

1. The CRF must be completed as soon as possible after the patient has been assessed or during the assessment if the CRF is the source data.
2. CRFs must be completed using a black ink ballpoint pen.
3. If the CRF is printed on carbonless duplication paper, a suitable separator must be inserted under the form being completed.
4. Data entry into the CRF must be complete as without omissions. If data are unavailable then 'unknown', 'missing', 'test not done' etc. should be inserted. The ambiguous phrase, 'not available' should be avoided.
5. All entries into the CRF must be accurate, legible and verifiable with the source data in the medical records (unless the CRF is the source data). Data must not be invented – this is fraud.

   N.B. Whenever a subject has been seen by clinical staff for the purposes of a clinical trial, the time, date and reason for visit must always be entered into the subject's corresponding hospital notes. Copies of trial investigations/results that are clinically significant or have an impact on the patient's clinical care must also be filed in the medical notes.
6. Any discrepancies between the CRF and the source data should be explained and the significance noted in the CRF and/or patient's medical records.
7. All CRF data derived from source documents must be transcribed exactly. This includes laboratory values, which unless otherwise agreed, should be entered without conversion from printed reports, even if, for multi-centre studies, the units of measurement differ from centre to centre.
8. For laboratory values that fall outside the laboratory's reference range or trial specific range or when a value shows a significant variation from one assessment to the next, this should be commented on and the significance noted in the CRF and/or patient's medical records.
9. The subject's identity should remain confidential at all times and as such the trial subject must only be identified in the CRF using a trial number or code.
10. Entries into the CRF must never be overwritten.
11. Corrections to the CRF must be made as follows:
    - An incorrect entry must be deleted with a single line through the text allowing the incorrect entry to remain legible. Correction fluid must never be used and entries must not be obliterated.
    - The correct data must be entered.
    - The correction must be initialled and dated and an explanation given of the correction, if applicable.
12. The CRF must be signed and dated where indicated, by the chief/principal investigator or designee (for example, research nurse at the end of an assessment) to assert that he/she believes the data is completed and correct.
13. All CRFs must be faxed/scanned weekly to Moorfields

Appendix Summary

Appendix 2 *No. of Unscheduled Visit Forms*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 3 *No. of Protocol Deviation Forms*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 4 *No. of Concomitant Medication Forms Prior to trial*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 5 *No. of Concomitant Medication Forms During the trial*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 6 *Early Withdrawal Form*	Yes ☐ No ☐
Appendix 7 *No. of Adverse Events Forms*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

PATIENT & PRIMARY REPAIR ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Patient Details	
Study Eye	Right ☐ Left ☐
Gender	Male ☐ Female ☐
Ethnic Origin	***Please complete Ethnic Categories Form***
Date of Birth *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Primary Repair Details	
Location of wound	Corneal ☐ Scleral/Corneal ☐ Scleral ☐
Anterior Vitrectomy	Yes ☐ No ☐
Location of Foreign Body	Yes ☐ No ☐
If Yes Specify	_____
Removal of Foreign Body	Yes ☐ No ☐
Classification of Trauma	Contusion ☐ Rupture ☐ Laceration ☐ Penetrating Injury ☐ Perforating Injury ☐
Date of Accident *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Date of Primary Repair *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __

Comments

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:	
Print Name:	**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

BASELINE ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Baseline Exam	
Date of Exam *(dd mm yyyy)*	___ ___ / ___ ___ / ___ ___ ___ ___
RAPD	Yes ☐ No ☐
AC Flare	0 ☐ + ☐ ++ ☐ +++ ☐ ++++ ☐ NP ☐
AC Cells	0 ☐ + ☐ ++ ☐ +++ ☐ ++++ ☐ NP ☐
Hyphaema	0 ☐ Microscopic ☐ <25% ☐ 25-50% ☐ 50-99% ☐ Total ☐
Iris *(Choose one)*	Normal ☐ Incomplete ☐ Incarcerated ☐ NP ☐
Lens *(Choose one)*	Clear ☐ AC IOL ☐ Aphakic ☐ Cataract ☐ PC IOL ☐ Traumatic Cataract ☐ NP ☐
Vit RPE Cells	0 ☐ + ☐ ++ ☐ +++ ☐ ++++ ☐ NP ☐
Vit Flare	0 ☐ + ☐ ++ ☐ +++ ☐ ++++ ☐ NP ☐
Vit Haemorrhage	0 ☐ + ☐ ++ ☐ +++ ☐ NP ☐
Vit Foreign Body *(Choose one)*	Intravitreal ☐ Intraretinal ☐ Intrascleral ☐ Retrobulbar ☐ Incarceration ☐ Not Present ☐
Clinical Diagnosis of Endophthalmitis	Yes ☐ No ☐
BCVA ETDRS (Enter no. of letters, r*ange 0-100)* If score '0' please specify	___ ___ ___ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-70)*	___ ___
Spherical Equivalent Dioptres *(Range – -40-40)*	___ ___ . ___ ___ NP ☐
Retinal Detachment *(If yes please complete the following section)*	Yes ☐ No ☐
If Yes Specify	Durations of RD (no. of days, Range 0-999 ___ ___ ___ Extent (clock hours) ___ ___ NP ☐
Macula Involved	Yes ☐ No ☐ NP ☐
Traction RD	Yes ☐ No ☐ NP ☐
PVR Present	Yes ☐ No ☐ NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)* A ☐ B ☐ C ☐ If grading C please specify *(range 1-12)* CA ☐ ___ ___ CP ☐ ___ ___
OCT	Yes ☐ No ☐ NP ☐
If Yes Specify	Macula Attachment Yes ☐ No ☐ NP ☐ Foveal Thickness *(Range 0-999)* ___ ___ ___ µm NP ☐ Macular Volume *(Range 0-100)* ___ ___ . ___ ___ mm^3 NP ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:	
Print Name:	**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

OPERATION RECORD FORM

Study Eye Data Only, all fields are mandatory

Operation Details	
Date of Operation *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Surgeon Grade	SPR ☐ Fellow ☐ Consultant ☐
Anaesthetic	Local ☐ General ☐
Operative Techniques *(Tick yes or no)*	Phako Yes ☐ No ☐; Vitrectomy Yes ☐ No ☐; Lensectomy Yes ☐ No ☐; Posterior Vit Detach Induced Yes ☐ No ☐; Heavy Liquid Yes ☐ No ☐; Membrane Peel Yes ☐ No ☐; Drainage Retinotomy Yes ☐ No ☐; Local Buckle Yes ☐ No ☐; Encircling Buckle Yes ☐ No ☐; Gas SF6 Yes ☐ No ☐; Gas C2F6 Yes ☐ No ☐; Gas C3F8 Yes ☐ No ☐; Oil (1000) Yes ☐ No ☐; Oil (5000) Yes ☐ No ☐; Oil (Heavy) Yes ☐ No ☐
Relaxing Retinectomy	Yes ☐ No ☐
If Yes Specify Degrees	__ __ __ °
Retinopexy *(Tick yes or no)*	Endolaser Yes ☐ No ☐; Indirect Laser Yes ☐ No ☐; Cryotherapy Yes ☐ No ☐
Complication Type *(Tick yes or no)*	Entry Site Breaks Yes ☐ No ☐; Choroidal Haemorrhage Yes ☐ No ☐; Subretinal Haemorrhage Yes ☐ No ☐; Preretinal Haemorrhage Yes ☐ No ☐; Haemorrhage at Retinectomy Yes ☐ No ☐; AC Haemorrhage Yes ☐ No ☐; Other Iatrogenic Breaks Yes ☐ No ☐; Lens Touch Yes ☐ No ☐; Deep Buckle Suture Yes ☐ No ☐; Failure to Reattach Retina Yes ☐ No ☐
Complication Other	Yes ☐ No ☐
If Yes Specify	_____ _____ _____
Adjunct given	Yes ☐ No ☐
PVR Present	Yes ☐ No ☐ NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)* A ☐ B ☐ C ☐ If grading C please specify *(range 1-12)* CA ☐ __ __ CP ☐ __ __

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:	
Print Name:	**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

RE-OPERATION RECORD FORM

Study Eye Data Only, all fields are mandatory

Re-Operation Details	
Date of Operation *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Surgeon Grade	SPR ☐ Fellow ☐ Consultant ☐
Anaesthetic	Local ☐ General ☐
Operative Techniques *(Tick yes or no)*	Phako Yes ☐ No ☐; Vitrectomy Yes ☐ No ☐; Lensectomy Yes ☐ No ☐; Posterior Vit Detach Induced Yes ☐ No ☐; Heavy Liquid Yes ☐ No ☐; Membrane Peel Yes ☐ No ☐; Drainage Retinotomy Yes ☐ No ☐; Local Buckle Yes ☐ No ☐; Encircling Buckle Yes ☐ No ☐; Gas SF6 Yes ☐ No ☐; Gas C2F6 Yes ☐ No ☐; Gas C3F8 Yes ☐ No ☐; Oil (1000) Yes ☐ No ☐; Oil (5000) Yes ☐ No ☐; Oil (Heavy) Yes ☐ No ☐
Relaxing Retinectomy	Yes ☐ No ☐
If Yes Specify Degrees	__ __ __ °
Retinopexy *(Tick yes or no)*	Endolaser Yes ☐ No ☐; Indirect Laser Yes ☐ No ☐; Cryotherapy Yes ☐ No ☐
Complication Type *(Tick yes or no)*	Entry Site Breaks Yes ☐ No ☐; Choroidal Haemorrhage Yes ☐ No ☐; Subretinal Haemorrhage Yes ☐ No ☐; Preretinal Haemorrhage Yes ☐ No ☐; Haemorrhage at Retinectomy Yes ☐ No ☐; AC Haemorrhage Yes ☐ No ☐; Other Iatrogenic Breaks Yes ☐ No ☐; Lens Touch Yes ☐ No ☐; Deep Buckle Suture Yes ☐ No ☐; Failure to Reattach Retina Yes ☐ No ☐
Complication Other	Yes ☐ No ☐
If Yes Specify	_____ _____ _____
Adjunct given	Yes ☐ No ☐
PVR Present	Yes ☐ No ☐ NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)* A ☐ B ☐ C ☐ If grading C please specify *(range 1-12)* CA ☐ __ __ CP ☐ __ __

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:	
Print Name:	**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

10 DAY ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
BCVA ETDRS (Enter no. of letters, range 0-100)	__ __ __
If score '0' please specify	CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-70)*	__ __

		Yes	No	NP		Yes	No	NP		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐	Fibrin	☐	☐	☐	Hyphaema	☐	☐	☐
	Oil (patent pi)	☐	☐	☐	Oil (blocked pi)	☐	☐	☐	Flat AC	☐	☐	☐
									Rubeosis	☐	☐	☐
Lens *(Tick yes or no)*	Clear	☐	☐	☐	Cataract (oil)	☐	☐	☐	Cataract	☐	☐	☐
	AC IOL	☐	☐	☐	PC IOL	☐	☐	☐	Aphakic	☐	☐	☐
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐	Detached	☐	☐	☐	Oedema	☐	☐	☐
					Pucker	☐	☐	☐	FTMH	☐	☐	☐
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐	Traction RD	☐	☐	☐	RRD	☐	☐	☐
	Open Previous Break	☐	☐	☐	Entry Site Breaks	☐	☐	☐	Other New Breaks	☐	☐	☐

Other Procedures Since Initial Surgery

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	__ __ / __ __ / __ __ __ __	Buckle	☐	☐	__ __ / __ __ / __ __ __ __
ICCE	☐	☐	__ __ / __ __ / __ __ __ __	Peel	☐	☐	__ __ / __ __ / __ __ __ __
E/C + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Retinectomy	☐	☐	__ __ / __ __ / __ __ __ __
Phako + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Laser	☐	☐	__ __ / __ __ / __ __ __ __
YAG PI	☐	☐	__ __ / __ __ / __ __ __ __	Surgical PI	☐	☐	__ __ / __ __ / __ __ __ __
Other	☐	☐	__ __ / __ __ / __ __ __ __				

If Other Specify _____

PVR Present		Yes ☐	No ☐	NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)*	A ☐	B ☐	C ☐
	If grading C please specify *(range 1-12)*	CA ☐ __ __	CP ☐ __ __	
OCT Taken		Yes ☐	No ☐	NP ☐
If Yes Specify	OCT Macular SRF	Yes ☐	No ☐	NP ☐
	OCT Foveal Thickness *(Range 0-999)*	__ __ __ µm		NP ☐
	OCT Macular Volume *(Range 0-100)*	__ __ . __ __ mm^3		NP ☐

Retina Re-Attached without further VR Intervention		Yes ☐	No ☐
Silicone Oil In situ		Yes ☐	No ☐
Number of Additional VR Procedures Since First Vitrectomy apart from removal of oil		__ __	N/A ☐
Any Adverse Events since their last study visit?	*(If yes complete separate adverse event form)*	Yes ☐	No ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

4-6 WEEK ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
BCVA ETDRS (Enter no. of letters, range 0-100)	__ __ __
If score '0' please specify	CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-70)*	__ __

AC *(Tick yes or no)*	Uveiitis Yes ☐ No ☐ NP ☐	Fibrin Yes ☐ No ☐ NP ☐	Hyphaema Yes ☐ No ☐ NP ☐
	Oil (patent pi) Yes ☐ No ☐ NP ☐	Oil (blocked pi) Yes ☐ No ☐ NP ☐	Flat AC Yes ☐ No ☐ NP ☐
			Rubeosis Yes ☐ No ☐ NP ☐
Lens *(Tick yes or no)*	Clear Yes ☐ No ☐ NP ☐	Cataract (oil) Yes ☐ No ☐ NP ☐	Cataract Yes ☐ No ☐ NP ☐
	AC IOL Yes ☐ No ☐ NP ☐	PC IOL Yes ☐ No ☐ NP ☐	Aphakic Yes ☐ No ☐ NP ☐
State of Macula *(Tick yes or no)*	Attached Yes ☐ No ☐ NP ☐	Detached Yes ☐ No ☐ NP ☐	Oedema Yes ☐ No ☐ NP ☐
		Pucker Yes ☐ No ☐ NP ☐	FTMH Yes ☐ No ☐ NP ☐
State of Retina *(Tick yes or no)*	Fully Attached Yes ☐ No ☐ NP ☐	Traction RD Yes ☐ No ☐ NP ☐	RRD Yes ☐ No ☐ NP ☐
	Open Previous Break Yes ☐ No ☐ NP ☐	Entry Site Breaks Yes ☐ No ☐ NP ☐	Other New Breaks Yes ☐ No ☐ NP ☐

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	__ __ / __ __ / __ __ __ __	Buckle	☐	☐	__ __ / __ __ / __ __ __ __
ICCE	☐	☐	__ __ / __ __ / __ __ __ __	Peel	☐	☐	__ __ / __ __ / __ __ __ __
E/C + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Retinectomy	☐	☐	__ __ / __ __ / __ __ __ __
Phako + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Laser	☐	☐	__ __ / __ __ / __ __ __ __
YAG PI	☐	☐	__ __ / __ __ / __ __ __ __	Surgical PI	☐	☐	__ __ / __ __ / __ __ __ __
Other	☐	☐	__ __ / __ __ / __ __ __ __				

If Other Specify _____

PVR Present	Yes ☐	No ☐	NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)*	A ☐	B ☐ C ☐
	If grading C please specify *(range 1-12)*	CA ☐ __ __	CP ☐ __ __
OCT Taken	Yes ☐	No ☐	NP ☐
If Yes Specify	OCT Macular SRF Yes ☐	No ☐	NP ☐
	OCT Foveal Thickness *(Range 0-999)*	__ __ __ µm	NP ☐
	OCT Macular Volume *(Range 0-100)*	__ __ . __ __ mm^3	NP ☐

Retina Re-Attached without further VR Intervention	Yes ☐	No ☐
Silicone Oil In situ	Yes ☐	No ☐
Number of Additional VR Procedures Since First Vitrectomy apart from removal of oil	__ __	N/A ☐
Any Adverse Events since their last study visit? *(If yes complete separate adverse event form)*	Yes ☐	No ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

3 MONTH ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
BCVA ETDRS (Enter no. of letters, range 0-100)	__ __ __
If score '0' please specify	CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-70)*	__ __

AC *(Tick yes or no)*	Uveiitis Yes ☐ No ☐ NP ☐	Fibrin Yes ☐ No ☐ NP ☐	Hyphaema Yes ☐ No ☐ NP ☐
	Oil (patent pi) Yes ☐ No ☐ NP ☐	Oil (blocked pi) Yes ☐ No ☐ NP ☐	Flat AC Yes ☐ No ☐ NP ☐
			Rubeosis Yes ☐ No ☐ NP ☐
Lens *(Tick yes or no)*	Clear Yes ☐ No ☐ NP ☐	Cataract (oil) Yes ☐ No ☐ NP ☐	Cataract Yes ☐ No ☐ NP ☐
	AC IOL Yes ☐ No ☐ NP ☐	PC IOL Yes ☐ No ☐ NP ☐	Aphakic Yes ☐ No ☐ NP ☐
State of Macula *(Tick yes or no)*	Attached Yes ☐ No ☐ NP ☐	Detached Yes ☐ No ☐ NP ☐	Oedema Yes ☐ No ☐ NP ☐
		Pucker Yes ☐ No ☐ NP ☐	FTMH Yes ☐ No ☐ NP ☐
State of Retina *(Tick yes or no)*	Fully Attached Yes ☐ No ☐ NP ☐	Traction RD Yes ☐ No ☐ NP ☐	RRD Yes ☐ No ☐ NP ☐
	Open Previous Break Yes ☐ No ☐ NP ☐	Entry Site Breaks Yes ☐ No ☐ NP ☐	Other New Breaks Yes ☐ No ☐ NP ☐

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	__ __ / __ __ / __ __ __ __	Buckle	☐	☐	__ __ / __ __ / __ __ __ __
ICCE	☐	☐	__ __ / __ __ / __ __ __ __	Peel	☐	☐	__ __ / __ __ / __ __ __ __
E/C + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Retinectomy	☐	☐	__ __ / __ __ / __ __ __ __
Phako + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Laser	☐	☐	__ __ / __ __ / __ __ __ __
YAG PI	☐	☐	__ __ / __ __ / __ __ __ __	Surgical PI	☐	☐	__ __ / __ __ / __ __ __ __
Other	☐	☐	__ __ / __ __ / __ __ __ __				

If Other Specify _____

PVR Present	Yes ☐	No ☐	NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)*	A ☐	B ☐ C ☐
	If grading C please specify *(range 1-12)*	CA ☐ __ __	CP ☐ __ __
OCT Taken	Yes ☐	No ☐	NP ☐
If Yes Specify	OCT Macular SRF Yes ☐	No ☐	NP ☐
	OCT Foveal Thickness *(Range 0-999)*	__ __ __ µm	NP ☐
	OCT Macular Volume *(Range 0-100)*	__ __ . __ __ mm^3	NP ☐

Retina Re-Attached without further VR Intervention	Yes ☐	No ☐
Silicone Oil In situ	Yes ☐	No ☐
Number of Additional VR Procedures Since First Vitrectomy apart from removal of oil	__ __	N/A ☐
Any Adverse Events since their last study visit?	*(If yes complete separate adverse event form)* Yes ☐	No ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

6 MONTH ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	___ ___ / ___ ___ / ___ ___ ___ ___
BCVA ETDRS (Enter no. of letters, *range 0-100*) If score '0' please specify	___ ___ ___ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-70)*	___ ___

AC *(Tick yes or no)*	Uveiitis Yes ☐ No ☐ NP ☐	Fibrin Yes ☐ No ☐ NP ☐	Hyphaema Yes ☐ No ☐ NP ☐
	Oil (patent pi) Yes ☐ No ☐ NP ☐	Oil (blocked pi) Yes ☐ No ☐ NP ☐	Flat AC Yes ☐ No ☐ NP ☐
			Rubeosis Yes ☐ No ☐ NP ☐
Lens *(Tick yes or no)*	Clear Yes ☐ No ☐ NP ☐	Cataract (oil) Yes ☐ No ☐ NP ☐	Cataract Yes ☐ No ☐ NP ☐
	AC IOL Yes ☐ No ☐ NP ☐	PC IOL Yes ☐ No ☐ NP ☐	Aphakic Yes ☐ No ☐ NP ☐
State of Macula *(Tick yes or no)*	Attached Yes ☐ No ☐ NP ☐	Detached Yes ☐ No ☐ NP ☐	Oedema Yes ☐ No ☐ NP ☐
		Pucker Yes ☐ No ☐ NP ☐	FTMH Yes ☐ No ☐ NP ☐
State of Retina *(Tick yes or no)*	Fully Attached Yes ☐ No ☐ NP ☐	Traction RD Yes ☐ No ☐ NP ☐	RRD Yes ☐ No ☐ NP ☐
	Open Previous Break Yes ☐ No ☐ NP ☐	Entry Site Breaks Yes ☐ No ☐ NP ☐	Other New Breaks Yes ☐ No ☐ NP ☐

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Buckle	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
ICCE	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Peel	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
E/C + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Retinectomy	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Phako + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Laser	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
YAG PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Surgical PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Other	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___				

If Other Specify _____

PVR Present		Yes ☐	No ☐	NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)*	A ☐	B ☐	C ☐
	If grading C please specify *(range 1-12)*	CA ☐ ___ ___	CP ☐ ___ ___	
OCT Taken		Yes ☐	No ☐	NP ☐
If Yes Specify	OCT Macular SRF	Yes ☐	No ☐	NP ☐
	OCT Foveal Thickness *(Range 0-999)*	___ ___ ___ µm		NP ☐
	OCT Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3		NP ☐
Retina Re-Attached without further VR Intervention			Yes ☐	No ☐
Silicone Oil In situ			Yes ☐	No ☐
Number of Additional VR Procedures Since First Vitrectomy apart from removal of oil		___ ___		N/A ☐
Any Adverse Events since their last study visit?	*(If yes complete separate adverse event form)*		Yes ☐	No ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

ETHNIC CATEGORIES FORM

Study Eye Data Only, all fields are mandatory

These are the standard categories to be used for the collection of ethnic group information from 1 April 2001.

Ethnic Categories	
White	British ☐ Irish ☐ Any other White background ☐
Mixed	White and Black Caribbean ☐ White and Black African ☐ White and Asian ☐ Any other mixed background ☐
Asian or Asian British	Indian ☐ Pakistani ☐ Bangladeshi ☐ Any other Asian background ☐
Black or Black British	Caribbean ☐ African ☐ Any other Black background ☐
Other ethnic groups	Chinese ☐ Any other ethnic group ☐
If Any Other Ethnic Group Specify	_____
Not stated	Not stated ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:	
Print Name:	**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

APPENDIX 1 - GRADING CHART

All fields are mandatory

PVR Grade	Features
A	Vitreous haze; vitreous pigment clumps; pigment clusters on inferior retina
B	Wrinkling of inner retinal surface; retinal stiffness; vessel tortousity; rolled and irregular edge of retinal break; decreased mobility of vitreous
CA 1-12	Anterior to equator; focal, diffuse or circumferential full thickness folds; subretinal strands; anterior displacement; condensed viterous with strands
CP 1-12	Posterior to equator; focal, diffuse or circumferential full-thickness folds; subretinal strands; anterior displacement; condensed vitreous with strands

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

APPENDIX 2 - UNSCHEDULED VISIT ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Date of Visit *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
BCVA ETDRS (Enter no. of letters, r*ange 0-100)* If score '0' please specify	__ __ __ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-70)*	__ __

AC *(Tick yes or no)*		*Yes No NP*	
	Uveiitis ☐ ☐ ☐	Fibrin ☐ ☐ ☐	Hyphaema ☐ ☐ ☐
	Oil (patent pi) ☐ ☐ ☐	Oil (blocked pi) ☐ ☐ ☐	Flat AC ☐ ☐ ☐
			Rubeosis ☐ ☐ ☐
Lens *(Tick yes or no)*		*Yes No NP*	
	Clear ☐ ☐ ☐	Cataract (oil) ☐ ☐ ☐	Cataract ☐ ☐ ☐
	AC IOL ☐ ☐ ☐	PC IOL ☐ ☐ ☐	Aphakic ☐ ☐ ☐
State of Macula *(Tick yes or no)*		*Yes No NP*	
	Attached ☐ ☐ ☐	Detached ☐ ☐ ☐	Oedema ☐ ☐ ☐
		Pucker ☐ ☐ ☐	FTMH ☐ ☐ ☐
State of Retina *(Tick yes or no)*		*Yes No NP*	
	Fully Attached ☐ ☐ ☐	Traction RD ☐ ☐ ☐	RRD ☐ ☐ ☐
	Open Previous Break ☐ ☐ ☐	Entry Site Breaks ☐ ☐ ☐	Other New Breaks ☐ ☐ ☐

Other Procedures Since Last Visit

	Yes	*No*	*(dd mm yyyy)*		*Yes*	*No*	*(dd mm yyyy)*
Oil Out	☐	☐	__ __ / __ __ / __ __ __ __	Buckle	☐	☐	__ __ / __ __ / __ __ __ __
ICCE	☐	☐	__ __ / __ __ / __ __ __ __	Peel	☐	☐	__ __ / __ __ / __ __ __ __
E/C + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Retinectomy	☐	☐	__ __ / __ __ / __ __ __ __
Phako + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Laser	☐	☐	__ __ / __ __ / __ __ __ __
YAG PI	☐	☐	__ __ / __ __ / __ __ __ __	Surgical PI	☐	☐	__ __ / __ __ / __ __ __ __
Other	☐	☐	__ __ / __ __ / __ __ __ __				

If Other Specify _____

PVR Present		Yes ☐	No ☐ NP ☐
If Yes Specify	PVR Grading *(See grading chart for further details)*	A ☐	B ☐ C ☐
	If grading C please specify *(range 1-12)*	CA ☐ __ __	CP ☐ __ __
OCT Taken		Yes ☐	No ☐ NP ☐
If Yes Specify	OCT Macular SRF	Yes ☐	No ☐ NP ☐
	OCT Foveal Thickness *(Range 0-999)*	__ __ __ µm	NP ☐
	OCT Macular Volume *(Range 0-100)*	__ __ . __ __ mm^3	NP ☐

Retina Re-Attached without further VR Intervention	Yes ☐	No ☐
Silicone Oil In situ	Yes ☐	No ☐
Number of Additional VR Procedures Since First Vitrectomy apart from removal of oil	__ __	N/A ☐
Any Adverse Events since their last study visit?	*(If yes complete separate adverse event form)* Yes ☐	No ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

APPENDIX 3 - PROTOCOL DEVIATIONS FORM

All fields are mandatory

Deviation Details	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:	
Print Name:	**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

APPENDIX 4 - CONCOMITANT MEDICATION FORM PRIOR TO TRIAL

All fields are mandatory

Name of Medication	**Total Dose** per day	Approximate **Start Date** of medication	**Stop Date** *(leave blank if continuing on the medication)*

Comments

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

APPENDIX 5 - CONCOMITANT MEDICATION FORM DURING THE TRIAL

All fields are mandatory

Name of Medication	**Total Dose** per day	Approximate **Start Date** of medication	**Stop Date** *(leave blank if continuing on the medication)*

Comments

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Study No: ___ ___ ___ ___

APPENDIX 6 – EARLY STUDY WITHDRAWAL FORM

All fields are mandatory

Withdrawal Details	
Did the patient discontinue the trial prematurely?	Yes ☐ No ☐
Date of premature Study Discontinuation *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Primary reason for discontinuation *(tick one box only)*	Patient withdraws consent ☐ *If known, state reason:* _____ Patient is participating in another trial ☐ Patient is non-compliant ☐ Patient is pregnant ☐ Patient is lost to follow up ☐ Investigator feels that it is in the patients best interest due to adverse event ☐ *Related AE No:* _____ Other reason for discontinuation ☐ *If Other specify:* _____
Does the patient still agree to have their data collected and analysed as part of an intent to treat analysis?	Yes ☐ No ☐

Comments

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: _____ **Print:** _____ **Date:** _____

Office use only, data entry completed by:	
Print Name:	**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study

Adverse Event Pharmacovigilance Log

Study No: ___ ___ ___ ___

Visit Number	Visit Date	Any Adverse Events since their last study visit
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐

Moorfields Eye Hospital NHS
NHS Foundation Trust

Triamcinolone at Vitrectomy for Trauma – AOT Study
APPENDIX 8 - ADVERSE EVENT FORM

Site number:	Sponsor ID:	Eudract Number:	Study Drug:	Patient Initials:	Visit Number:
MEH/001	CHAD1024	2007/005138/55	Triamcinolone, Pred Forte, Froben		

Adverse Event Ocular/Non-ocular	Severity	Study Drug Relationship	Action Taken Regarding Study Treatment	Outcome of AE	Expected	Serious
L=event in left eye R=event in right eye B=event in both eyes N=Other non-ocular event (code each prefix/ non event)	1=Mild 2=Moderate 3=Severe	1=Definitely 2=Probably 3=Possibly 4=Unlikely 5=Not Related 6=Not Assessable	1=None 2=Discontinued permanently 3=Discontinued temporarily 4=Reduced dose 5=Increased dose 6=Delayed dose	1=Resolved, No Sequel 2=AE still present- no treatment 3=AE still present- being treated 4=Side effects present- not treated 5=Side effects present- treated 6=Death 7=Unknown	1=Yes 2=No	1=Yes 2=No ***(If yes complete SAE form)***

Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious *(If yes complete SAE form)*	Initials
1.									
2.									
3.									

8.2 Appendix 2 Adjuncts in Ocular Trauma Trial GP Letter

Dear Doctor

This is to inform you that your patient:

has been recruited into a new study at Moorfields Eye Hospital entitled:

A pilot study of intraocular use of intensive anti-inflammatory; Triamcinolone Acetonide to prevent proliferative vitreoretinopathy (PVR) in eyes undergoing vitreoretinal surgery for open globe trauma (OGT)

Please find enclosed a copy of the Participant Information Sheet.

Your patient has given written consent to take part in this study. They are of course free to withdraw at any time, without needing to give a reason. The study has been subject to a review by The Research Ethics Committee for Wales, who have given approval for the study to take place.

If you require any further information or you feel that your patient is unsuitable to take part in this trial, please do not hesitate to contact us.

Yours sincerely

8.3 Appendix 3 Adjuncts in Ocular Trauma Trial Consent Form

CONSENT FORM

Study Title: A pilot study of intraocular use of intensive anti-inflammatory; Triamcinolone Acetonide to prevent proliferative vitreoretinopathy (PVR) in eyes undergoing vitreoretinal surgery for open globe trauma (OGT)

Researcher: Mr D. G. Charteris

Please initial box

1. I confirm that I have read and understand the information sheet dated July 2011 version number 1.5 for the above study and have had the opportunity to ask questions ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records ☐

4. I understand that my GP will be informed of my participation in this research project and of any findings significant to my general health ☐

5. I agree to take part in the above study ☐

_____	_____	_____
Name of patient	**Date**	**Signature**
_____	_____	_____
Name of person taking consent (if different from researcher)	**Date**	**Signature**
_____	_____	_____
Researcher	**Date**	**Signature**

8.4 Appendix 4 Ozurdex in PVR Case Report Form Pack

Moorfields Eye Hospital NHS
NHS Foundation Trust

Completing Case Report Forms (CRFs)

This document has been created to provide guidelines about completing clinical trial case report forms at Moorfields Eye Hospital (MEH). The information has been extracted from the revised MEH SOP documents that are being developed by the Research & Development department at Moorfields.

1. The CRF must be completed as soon as possible after the patient has been assessed or during the assessment if the CRF is the source data.
2. CRFs must be completed using a black ink ballpoint pen.
3. If the CRF is printed on carbonless duplication paper, a suitable separator must be inserted under the form being completed.
4. Data entry into the CRF must be complete as without omissions. If data are unavailable then 'unknown', 'missing', 'test not done' etc. should be inserted. The ambiguous phrase, 'not available' should be avoided.
5. All entries into the CRF must be accurate, legible and verifiable with the source data in the medical records (unless the CRF is the source data). Data must not be invented – this is fraud.

   N.B. Whenever a subject has been seen by clinical staff for the purposes of a clinical trial, the time, date and reason for visit must always be entered into the subject's corresponding hospital notes. Copies of trial investigations/results that are clinically significant or have an impact on the patient's clinical care must also be filed in the medical notes.
6. Any discrepancies between the CRF and the source data should be explained and the significance noted in the CRF and/or patient's medical records.
7. All CRF data derived from source documents must be transcribed exactly. This includes laboratory values, which unless otherwise agreed, should be entered without conversion from printed reports, even if, for multi-centre studies, the units of measurement differ from centre to centre.
8. For laboratory values that fall outside the laboratory's reference range or trial specific range or when a value shows a significant variation from one assessment to the next, this should be commented on and the significance noted in the CRF and/or patient's medical records.
9. The subject's identity should remain confidential at all times and as such the trial subject must only be identified in the CRF using a trial number or code.
10. Entries into the CRF must never be overwritten.
11. Corrections to the CRF must be made as follows:
    - An incorrect entry must be deleted with a single line through the text allowing the incorrect entry to remain legible. Correction fluid must never be used and entries must not be obliterated.
    - The correct data must be entered.
    - The correction must be initialled and dated and an explanation given of the correction, if applicable.
12. The CRF must be signed and dated where indicated, by the chief/principal investigator or designee (for example, research nurse at the end of an assessment) to assert that he/she believes the data is completed and correct.
13. All CRFs must be faxed/scanned weekly to Moorfields

Appendix Summary

Appendix 2 Patient Questionnaire Forms Completed	Depression Screener ☐ SF36 ☐ VFQ-25 ☐
Appendix 3 *No. of Unscheduled Visit Forms*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 4 *No. of Protocol Deviation Forms*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 5 *No. of Concomitant Medication Forms Prior to trial*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 6 *No. of Concomitant Medication Forms During the trial*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐
Appendix 7 *Early Withdrawal Form*	Yes ☐ No ☐
Appendix 8 *No. of Adverse Events Forms*	0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

PATIENT & BASELINE ASSESSMENT FORM (1/2)

Study Eye Data Only, all fields are mandatory

Patient Details	
Study Eye	Right ☐ Left ☐
Gender	Male ☐ Female ☐
Ethnic Origin	***Please complete Ethnic Categories Form***
Date of Birth *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Baseline Exam	
Date of Exam *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
VFQ score *(Range – 0-100)*	__ __ __
SF36 score *(Range – 0-100)*	__ __ __
Dysthmia/ Major Depression	Yes ☐ No ☐
If yes are symptoms present?	Yes ☐ No ☐
Spherical Equivalent Dioptres *(Range – -30-30)*	+/- __ __ . __ __ NP ☐
Past Ocular History	Uveitis Yes ☐ No ☐ Previous VR surgery Yes ☐ No ☐ Stable glaucoma/OHT Yes ☐ No ☐ Other *(except cataract surgery)* Yes ☐ No ☐
If Other Specify	_____
If Previous VR Surgery Specify	*Yes* *No* *(dd mm yyyy)* V/Gas ☐ ☐ __ __ / __ __ / __ __ __ __ V/Oil ☐ ☐ __ __ / __ __ / __ __ __ __ V/B ☐ ☐ __ __ / __ __ / __ __ __ __ C/B ☐ ☐ __ __ / __ __ / __ __ __ __ Other ☐ ☐ __ __ / __ __ / __ __ __ __
If Other Specify	_____
Extent of Buckle (hrs 0-12)	__ __
No' of times Mac off	__ __
ETDRS VA (Enter no. of letters, r*ange 0-100)* If score '0' please specify	__ __ __ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	__ __
AC Flare	0 ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐ NP ☐
AC Cells	0 ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐ NP ☐
Lens Status *(Choose one)*	Clear ☐ PC IOL ☐ Cataract ☐ Traumatic Cataract ☐ NP ☐
If Cataract, state LOCS II	NS 0 ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐ CLO 0 ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐ 5+ ☐ PSC 0 ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐
Vit Haemorrhage	0 ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐ NP ☐
Vit RPE Cells	0 ☐ 1+ ☐ 2+ ☐ 3+ ☐ 4+ ☐ NP ☐

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

PATIENT & BASELINE ASSESSMENT FORM (2/2)

Study Eye Data Only, all fields are mandatory

		Primary	*Baseline*
Retinal Detachment *(Please enter Primary if first Detachment or enter both Primary and Baseline if more than one Detachment)*	Number of Breaks	___ ___ ___ NP ☐	___ ___ ___ NP ☐
	Clock hours of Breaks	___ ___ ___ NP ☐	___ ___ ___ NP ☐
	Duration of RD	___ ___ ___ NP ☐	___ ___ ___ NP ☐
	Clock hours of RD	___ ___ ___ NP ☐	___ ___ ___ NP ☐
Macula Attached		Yes ☐ No ☐	Bisecting ☐
OCT		Yes ☐ No ☐	NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	___ ___ ___ µm	NP ☐
	Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3	NP ☐
PVR Grade *(range 1-12)*		CA ☐ ___ ___	CP ☐ ___ ___

Comments

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed:	**Print:**	**Date:**
Office use only, data entry completed by:		
Print Name:		**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

OPERATION RECORD FORM

Study Eye Data Only, all fields are mandatory

Operation Details	
Date of Operation *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Surgeon Grade	SPR ☐ Fellow ☐ Consultant ☐
Anaesthetic	Local ☐ General ☐
Operative Techniques *(Tick yes or no)*	Phako Yes ☐ No ☐; Vitrectomy Yes ☐ No ☐; Lensectomy Yes ☐ No ☐; Posterior Vit Detach Induced Yes ☐ No ☐; Heavy Liquid Yes ☐ No ☐; Drainage Retinotomy Yes ☐ No ☐; Encircling Buckle Yes ☐ No ☐; Oil (1300) Yes ☐ No ☐; Oil (5500) Yes ☐ No ☐
Relaxing Retinectomy	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Peel	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Local Buckle	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Retinopexy *(Tick yes or no)*	Endolaser Yes ☐ No ☐; Indirect Laser Yes ☐ No ☐; Cryotherapy Yes ☐ No ☐
Complication Type *(Tick yes or no)*	Entry Site Breaks Yes ☐ No ☐; Choroidal Haemorrhage Yes ☐ No ☐; Subretinal Haemorrhage Yes ☐ No ☐; Preretinal Haemorrhage Yes ☐ No ☐; Haemorrhage at Retinectomy Yes ☐ No ☐; AC Haemorrhage Yes ☐ No ☐; Other Iatrogenic Breaks Yes ☐ No ☐; Lens Touch Yes ☐ No ☐; Deep Buckle Suture Yes ☐ No ☐
Failure to Reattach Retina	Yes ☐ No ☐
Complication Other	Yes ☐ No ☐
If Yes Specify	_____
PVR Present	Yes ☐ No ☐
If Yes Specify Grade (range 1-12)	CA ☐ __ __ CP ☐ __ __
Adjunct given	Yes ☐ No ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

RE-OPERATION RECORD FORM

Study Eye Data Only, all fields are mandatory

Operation Details	
Date of Operation *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Surgeon Grade	SPR ☐ Fellow ☐ Consultant ☐
Anaesthetic	Local ☐ General ☐
Operative Techniques *(Tick yes or no)*	Phako Yes ☐ No ☐; Vitrectomy Yes ☐ No ☐; Lensectomy Yes ☐ No ☐; Posterior Vit Detach Induced Yes ☐ No ☐; Heavy Liquid Yes ☐ No ☐; Drainage Retinotomy Yes ☐ No ☐; Encircling Buckle Yes ☐ No ☐; Oil (1300) Yes ☐ No ☐; Oil (5500) Yes ☐ No ☐
Relaxing Retinectomy	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Peel	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Local Buckle	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Retinopexy *(Tick yes or no)*	Endolaser Yes ☐ No ☐; Indirect Laser Yes ☐ No ☐; Cryotherapy Yes ☐ No ☐
Complication Type *(Tick yes or no)*	Entry Site Breaks Yes ☐ No ☐; Choroidal Haemorrhage Yes ☐ No ☐; Subretinal Haemorrhage Yes ☐ No ☐; Preretinal Haemorrhage Yes ☐ No ☐; Haemorrhage at Retinectomy Yes ☐ No ☐; AC Haemorrhage Yes ☐ No ☐; Other Iatrogenic Breaks Yes ☐ No ☐; Lens Touch Yes ☐ No ☐; Deep Buckle Suture Yes ☐ No ☐
Failure to Reattach Retina	Yes ☐ No ☐
Complication Other	Yes ☐ No ☐
If Yes Specify	_____
PVR Present	Yes ☐ No ☐
If Yes Specify Grade (range 1-12)	CA ☐ __ __ CP ☐ __ __

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

Office use only, data entry completed by:	
Print Name:	**Date:**

Ozurdex in PVR Study

Study No: ___ ___ ___

REMOVAL OF SILICONE OIL PROCEDURE FORM (1/2)

Study Eye Data Only, all fields are mandatory

Removal of Silicone Oil Procedure	
Date of Operation *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Surgeon Grade	SPR ☐ Fellow ☐ Consultant ☐
Anaesthetic	Local ☐ General ☐
State of Retina *(Tick yes or no)*	Fully Attached: Yes ☐ No ☐ NP ☐ · Traction RD: Yes ☐ No ☐ NP ☐ · RRD: Yes ☐ No ☐ NP ☐ · Open Previous Break: Yes ☐ No ☐ NP ☐ · Entry Site Break: Yes ☐ No ☐ NP ☐ · Other New Breaks: Yes ☐ No ☐ NP ☐ · Visible Flat ERM: Yes ☐ No ☐ NP ☐
Macula Attached	Yes ☐ No ☐ Bisecting ☐
Operative Techniques *(Tick yes or no)*	Cataract Extraction: Yes ☐ No ☐ · Light Shield Used: Yes ☐ No ☐
Relaxing Retinectomy	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Peel	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Local Buckle	Yes ☐ No ☐
If Yes Specify Degrees (range 1-360)	__ __ __ °
Retinopexy *(Tick yes or no)*	Endolaser: Yes ☐ No ☐ · Indirect Laser: Yes ☐ No ☐ · Cryotherapy: Yes ☐ No ☐
Infusion site	AC ☐ Pars Plana ☐
IOL	Yes ☐ No ☐
If Yes Specify	AC IOL ☐ Sulcus ☐ BAG ☐
Evacuation Method	Automated ☐ Manual ☐
Evacuation Site	AC ☐ Pars Plana ☐
Tamponade	Yes ☐ No ☐
If Yes Specify	Air ☐ SF6 ☐ C3F8 ☐ Oil 1300 ☐ Oil 5500 ☐
Complication Type *(Tick yes or no)*	Entry Site Breaks: Yes ☐ No ☐ · Choroidal Haemorrhage: Yes ☐ No ☐ · Subretinal Haemorrhage: Yes ☐ No ☐ · Preretinal Haemorrhage: Yes ☐ No ☐ · Haemorrhage at Retinectomy: Yes ☐ No ☐ · AC Haemorrhage: Yes ☐ No ☐ · Other Iatrogenic Breaks: Yes ☐ No ☐ · Lens Touch: Yes ☐ No ☐ · Deep Buckle Suture: Yes ☐ No ☐
Failure to Reattach Retina	Yes ☐ No ☐

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

REMOVAL OF SILICONE OIL PROCEDURE FORM (1/2)

Study Eye Data Only, all fields are mandatory

Complication Other	Yes ☐ No ☐
If Yes Specify	_____
PVR Present	Yes ☐ No ☐
If Yes Specify Grade *(range 1-12)*	CA ☐ __ __ CP ☐ __ __
Adjunct given	Yes ☐ No ☐
Duration of Operation *(range 0-240 mins)*	__ __ __ . __ **mins**

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed:	**Print:**	**Date:**
Office use only, data entry completed by:		
Print Name:		**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

10 DAY ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
ETDRS VA *(Enter no. of letters, range 0-100)*	___ ___ ___
If score '0' please specify	CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	___ ___

		Yes	No	NP		Yes	No	NP		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐	Oil (patent PI)	☐	☐	☐	Corneal Oedema	☐	☐	☐
	Fibrin	☐	☐	☐	Oil (blocked PI)	☐	☐	☐				
	Hyphaema	☐	☐	☐	Rubeosis	☐	☐	☐				
Lens *(Tick yes or no)*	Clear	☐	☐	☐	PC IOL	☐	☐	☐	Cataract	☐	☐	☐
	AC IOL	☐	☐	☐	Aphakic	☐	☐	☐				

If Cataract, state LOCS II	NS		0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	CLO	0 ☐		1+ ☐	2+ ☐	3+ ☐	4+ ☐	5+ ☐
	PSC			0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐

		Yes	No	NP		Yes	No	NP		Yes	No	NP
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐	Oedema	☐	☐	☐	FTMH	☐	☐	☐
	Detached	☐	☐	☐	Pucker	☐	☐	☐				
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐	Open Previous Break	☐	☐	☐	Visible Flat ERM	☐	☐	☐
	Traction RD	☐	☐	☐	Entry Site Break	☐	☐	☐				
	RRD	☐	☐	☐	Other New Breaks	☐	☐	☐				

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	__ __ / __ __ / __ __ __ __	Buckle	☐	☐	__ __ / __ __ / __ __ __ __
ICCE	☐	☐	__ __ / __ __ / __ __ __ __	Peel	☐	☐	__ __ / __ __ / __ __ __ __
E/C + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Retinectomy	☐	☐	__ __ / __ __ / __ __ __ __
Phako + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Laser	☐	☐	__ __ / __ __ / __ __ __ __
YAG PI	☐	☐	__ __ / __ __ / __ __ __ __	Surgical PI	☐	☐	__ __ / __ __ / __ __ __ __
Other	☐	☐	__ __ / __ __ / __ __ __ __	*If Other Specify*			_____

OCT	Yes ☐	No ☐	NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	___ ___ ___ µm	NP ☐
	Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3	NP ☐
PVR Present	Yes ☐		No ☐
If Yes Specify Grade (range 1-12)	CA ☐ __ __		CP ☐ __ __
Hypotensive Agents	Yes ☐		No ☐
If Yes Specify	Prostaglandin ☐	Topical CAI ☐	Combigan ☐
	B Blocker ☐	Systemic CAI ☐	Combined B Blocker + Prostaglandin ☐
	Alpha Agonist ☐	Cosopt ☐	

Retina Reattached without further VR surgical intervention	Yes ☐	No ☐
Silicone Oil In situ	Yes ☐	No ☐
Number of additional VR procedures since first vitrectomy apart from ROSO		__ __

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

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Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

4-6 WEEK ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	___ ___ / ___ ___ / ___ ___ ___ ___
ETDRS VA *(Enter no. of letters, range 0-100)* If score '0' please specify	___ ___ ___ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	___ ___

		Yes	No	NP		Yes	No	NP		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐	Oil (patent PI)	☐	☐	☐	Corneal Oedema	☐	☐	☐
	Fibrin	☐	☐	☐	Oil (blocked PI)	☐	☐	☐				
	Hyphaema	☐	☐	☐	Rubeosis	☐	☐	☐				
Lens *(Tick yes or no)*	Clear	☐	☐	☐	PC IOL	☐	☐	☐	Cataract	☐	☐	☐
	AC IOL	☐	☐	☐	Aphakic	☐	☐	☐				

If Cataract, state LOCS II	NS	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	CLO	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐	5+ ☐
	PSC	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐

		Yes	No	NP		Yes	No	NP		Yes	No	NP
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐	Oedema	☐	☐	☐	FTMH	☐	☐	☐
	Detached	☐	☐	☐	Pucker	☐	☐	☐				
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐	Open Previous Break	☐	☐	☐	Visible Flat ERM	☐	☐	☐
	Traction RD	☐	☐	☐	Entry Site Break	☐	☐	☐				
	RRD	☐	☐	☐	Other New Breaks	☐	☐	☐				

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Buckle	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
ICCE	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Peel	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
E/C + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Retinectomy	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Phako + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Laser	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
YAG PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Surgical PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Other	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	*If Other Specify*	_____		

OCT	Yes ☐	No ☐	NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	___ ___ ___ µm	NP ☐
	Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3	NP ☐
PVR Present	Yes ☐	No ☐	
If Yes Specify Grade (range 1-12)	CA ☐ ___ ___	CP ☐ ___ ___	
Hypotensive Agents	Yes ☐	No ☐	
If Yes Specify	Prostaglandin ☐	Topical CAI ☐	Combigan ☐
	B Blocker ☐	Systemic CAI ☐	Combined B Blocker + Prostaglandin ☐
	Alpha Agonist ☐	Cosopt ☐	
Retina Reattached without further VR surgical intervention	Yes ☐	No ☐	
Silicone Oil In situ	Yes ☐	No ☐	
Number of additional VR procedures since first vitrectomy apart from ROSO	___ ___		

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

3 MONTH ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
ETDRS VA *(Enter no. of letters, range 0-100)* If score '0' please specify	___ ___ ___ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	___ ___

		Yes	No	NP		Yes	No	NP		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐	Oil (patent PI)	☐	☐	☐	Corneal Oedema	☐	☐	☐
	Fibrin	☐	☐	☐	Oil (blocked PI)	☐	☐	☐				
	Hyphaema	☐	☐	☐	Rubeosis	☐	☐	☐				
Lens *(Tick yes or no)*	Clear	☐	☐	☐	PC IOL	☐	☐	☐	Cataract	☐	☐	☐
	AC IOL	☐	☐	☐	Aphakic	☐	☐	☐				

If Cataract, state LOCS II	NS	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	CLO		2+ ☐	3+ ☐	4+ ☐	5+ ☐
	PSC		1+ ☐	2+ ☐	3+ ☐	4+ ☐

		Yes	No	NP		Yes	No	NP		Yes	No	NP
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐	Oedema	☐	☐	☐	FTMH	☐	☐	☐
	Detached	☐	☐	☐	Pucker	☐	☐	☐				
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐	Open Previous Break	☐	☐	☐	Visible Flat ERM	☐	☐	☐
	Traction RD	☐	☐	☐	Entry Site Break	☐	☐	☐				
	RRD	☐	☐	☐	Other New Breaks	☐	☐	☐				

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	__ __ / __ __ / __ __ __ __	Buckle	☐	☐	__ __ / __ __ / __ __ __ __
ICCE	☐	☐	__ __ / __ __ / __ __ __ __	Peel	☐	☐	__ __ / __ __ / __ __ __ __
E/C + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Retinectomy	☐	☐	__ __ / __ __ / __ __ __ __
Phako + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Laser	☐	☐	__ __ / __ __ / __ __ __ __
YAG PI	☐	☐	__ __ / __ __ / __ __ __ __	Surgical PI	☐	☐	__ __ / __ __ / __ __ __ __
Other	☐	☐	__ __ / __ __ / __ __ __ __	*If Other Specify*			_____

OCT	Yes ☐	No ☐	NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	___ ___ ___ µm	NP ☐
	Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3	NP ☐
PVR Present	Yes ☐		No ☐
If Yes Specify Grade (range 1-12)	CA ☐ __ __		CP ☐ __ __
Hypotensive Agents	Yes ☐		No ☐
If Yes Specify	Prostaglandin ☐	Topical CAI ☐	Combigan ☐
	B Blocker ☐	Systemic CAI ☐	Combined B Blocker + Prostaglandin ☐
	Alpha Agonist ☐	Cosopt ☐	

Retina Reattached without further VR surgical intervention	Yes ☐	No ☐
Silicone Oil In situ	Yes ☐	No ☐
Number of additional VR procedures since first vitrectomy apart from ROSO		__ __

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

6 MONTH ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	___ ___ / ___ ___ / ___ ___ ___ ___
ETDRS VA *(Range 0-100)* If score '0' please specify	___ ___ ___ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	___ ___

		Yes	No	NP		Yes	No	NP		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐	Oil (patent PI)	☐	☐	☐	Corneal Oedema	☐	☐	☐
	Fibrin	☐	☐	☐	Oil (blocked PI)	☐	☐	☐				
	Hyphaema	☐	☐	☐	Rubeosis	☐	☐	☐				
Lens *(Tick yes or no)*	Clear	☐	☐	☐	PC IOL	☐	☐	☐	Cataract	☐	☐	☐
	AC IOL	☐	☐	☐	Aphakic	☐	☐	☐				

If Cataract, state LOCS II	NS	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	CLO	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	PSC	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐

		Yes	No	NP		Yes	No	NP		Yes	No	NP
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐	Oedema	☐	☐	☐	FTMH	☐	☐	☐
	Detached	☐	☐	☐	Pucker	☐	☐	☐				
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐	Open Previous Break	☐	☐	☐	Visible Flat ERM	☐	☐	☐
	Traction RD	☐	☐	☐	Entry Site Break	☐	☐	☐				
	RRD	☐	☐	☐	Other New Breaks	☐	☐	☐				

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Buckle	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
ICCE	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Peel	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
E/C + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Retinectomy	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Phako + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Laser	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
YAG PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Surgical PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Other	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	*If Other Specify*	_____		

OCT		Yes ☐	No ☐ NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	___ ___ ___ µm	NP ☐
	Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3	NP ☐
PVR Taken		Yes ☐	No ☐
If Yes Specify Grade (range 1-12)		CA ☐ ___ ___	CP ☐ ___ ___
Hypotensive Agents		Yes ☐	No ☐
If Yes Specify	Prostaglandin ☐	Topical CAI ☐	Combigan ☐
	B Blocker ☐	Systemic CAI ☐	Combined B Blocker + Prostaglandin ☐
	Alpha Agonist ☐	Cosopt ☐	
VFQ score *(Range – 0-100)*			___ ___ ___
SF36 score *(Range – 0-100)*			___ ___ ___
Dysthmia/ Major Depression		Yes ☐	No ☐
If yes are symptoms present?		Yes ☐	No ☐
Retina Reattached without further VR surgical intervention		Yes ☐	No ☐
Silicone Oil In situ		Yes ☐	No ☐
Number of additional VR procedures since first vitrectomy apart from ROSO			___ ___

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

9 MONTH ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	___ ___ / ___ ___ / ___ ___ ___ ___
ETDRS VA *(Enter no. of letters, range 0-100)* If score '0' please specify	___ ___ ___ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	___ ___

		Yes	No	NP		Yes	No	NP		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐	Oil (patent PI)	☐	☐	☐	Corneal Oedema	☐	☐	☐
	Fibrin	☐	☐	☐	Oil (blocked PI)	☐	☐	☐				
	Hyphaema	☐	☐	☐	Rubeosis	☐	☐	☐				
Lens *(Tick yes or no)*	Clear	☐	☐	☐	PC IOL	☐	☐	☐	Cataract	☐	☐	☐
	AC IOL	☐	☐	☐	Aphakic	☐	☐	☐				

If Cataract, state LOCS II	NS	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	CLO	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	PSC	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐

		Yes	No	NP		Yes	No	NP		Yes	No	NP
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐	Oedema	☐	☐	☐	FTMH	☐	☐	☐
	Detached	☐	☐	☐	Pucker	☐	☐	☐				
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐	Open Previous Break	☐	☐	☐	Visible Flat ERM	☐	☐	☐
	Traction RD	☐	☐	☐	Entry Site Break	☐	☐	☐				
	RRD	☐	☐	☐	Other New Breaks	☐	☐	☐				

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Buckle	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
ICCE	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Peel	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
E/C + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Retinectomy	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Phako + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Laser	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
YAG PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Surgical PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Other	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	*If Other Specify*			

OCT	Yes ☐	No ☐	NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	___ ___ ___ µm	NP ☐
	Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3	NP ☐
PVR Present	Yes ☐		No ☐
If Yes Specify Grade (range 1-12)	CA ☐ ___ ___		CP ☐ ___ ___
Hypotensive Agents	Yes ☐		No ☐
If Yes Specify	Prostaglandin ☐	Topical CAI ☐	Combigan ☐
	B Blocker ☐	Systemic CAI ☐	Combined B Blocker + Prostaglandin ☐
	Alpha Agonist ☐	Cosopt ☐	

Retina Reattached without further VR surgical intervention	Yes ☐	No ☐
Silicone Oil In situ	Yes ☐	No ☐
Number of additional VR procedures since first vitrectomy apart from ROSO	___ ___	

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

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Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

12 MONTH ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	___ ___ / ___ ___ / ___ ___ ___ ___
ETDRS VA *(Range 0-100)* If score '0' please specify	___ ___ ___ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	___ ___

		Yes	No	NP		Yes	No	NP		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐	Oil (patent PI)	☐	☐	☐	Corneal Oedema	☐	☐	☐
	Fibrin	☐	☐	☐	Oil (blocked PI)	☐	☐	☐				
	Hyphaema	☐	☐	☐	Rubeosis	☐	☐	☐				
Lens *(Tick yes or no)*	Clear	☐	☐	☐	PC IOL	☐	☐	☐	Cataract	☐	☐	☐
	AC IOL	☐	☐	☐	Aphakic	☐	☐	☐				

If Cataract, state LOCS II	NS	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	CLO	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	PSC	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐

		Yes	No	NP		Yes	No	NP		Yes	No	NP
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐	Oedema	☐	☐	☐	FTMH	☐	☐	☐
	Detached	☐	☐	☐	Pucker	☐	☐	☐				
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐	Open Previous Break	☐	☐	☐	Visible Flat ERM	☐	☐	☐
	Traction RD	☐	☐	☐	Entry Site Break	☐	☐	☐				
	RRD	☐	☐	☐	Other New Breaks	☐	☐	☐				

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Buckle	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
ICCE	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Peel	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
E/C + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Retinectomy	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Phako + IOL	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Laser	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
YAG PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	Surgical PI	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___
Other	☐	☐	___ ___ / ___ ___ / ___ ___ ___ ___	*If Other Specify*	_____		

OCT	Yes ☐	No ☐	NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	___ ___ ___ µm	NP ☐
	Macular Volume *(Range 0-100)*	___ ___ . ___ ___ mm^3	NP ☐
PVR Taken	Yes ☐		No ☐
If Yes Specify Grade (range 1-12)	CA ☐ ___ ___		CP ☐ ___ ___
Hypotensive Agents	Yes ☐		No ☐
If Yes Specify	Prostaglandin ☐	Topical CAI ☐	Combigan ☐
	B Blocker ☐	Systemic CAI ☐	Combined B Blocker + Prostaglandin ☐
	Alpha Agonist ☐	Cosopt ☐	
VFQ score *(Range – 0-100)*			___ ___ ___
SF36 score *(Range – 0-100)*			___ ___ ___
Dysthmia/ Major Depression		Yes ☐	No ☐
If yes are symptoms present?		Yes ☐	No ☐
Retina Reattached without further VR surgical intervention		Yes ☐	No ☐
Silicone Oil In situ		Yes ☐	No ☐
Number of additional VR procedures since first vitrectomy apart from ROSO			___ ___

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed: **Print:** **Date:**

Office use only, data entry completed by:

Print Name: **Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

INTERIM ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Day 1 Details (Post Op primary study vitrectomy)	*Missed Visit*? ☐
Date of Visit *(dd mm yyyy)*	__ __/__ __/__ __ __ __
IOP mmHG *(Range – 0-80)*	__ __
If treatment group is implant in vitreous cavity?	Yes ☐ No ☐ Adjunct not given ☐
Day 60 Details (Post primary study vitrectomy)	*Missed Visit*? ☐
Date of Visit *(dd mm yyyy)*	__ __/__ __/__ __ __ __
IOP mmHG *(Range – 0-80)*	__ __
ETDRS VA (Enter no. of letters, range 0-100)	__ __ __
If score '0' please specify	CF ☐ HM ☐ PL ☐ NPL ☐
Day 1 Details (Post Op ROSO)	*Missed Visit*? ☐
Date of Visit *(dd mm yyyy)*	__ __/__ __/__ __ __ __
IOP mmHG *(Range – 0-80)*	__ __
If treatment group is implant in vitreous cavity?	Yes ☐ No ☐ Adjunct not given ☐
Day 60 Details (Post ROSO)	*Missed Visit*? ☐
Date of Visit *(dd mm yyyy)*	__ __/__ __/__ __ __ __
IOP mmHG *(Range – 0-80)*	__ __
ETDRS VA (Enter no. of letters, range 0-100)	__ __ __
If score '0' please specify	CF ☐ HM ☐ PL ☐ NPL ☐

Comments

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed:	**Print:**	**Date:**
Office use only, data entry completed by:		
Print Name:		**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

ETHNIC CATEGORIES FORM

Study Eye Data Only, all fields are mandatory

These are the standard categories to be used for the collection of ethnic group information from 1 April 2001.

Ethnic Categories	
White	British ☐ Irish ☐ Any other White background ☐
Mixed	White and Black Caribbean ☐ White and Black African ☐ White and Asian ☐ Any other mixed background ☐
Asian or Asian British	Indian ☐ Pakistani ☐ Bangladeshi ☐ Any other Asian background ☐
Black or Black British	Caribbean ☐ African ☐ Any other Black background ☐
Other ethnic groups	Chinese ☐ Any other ethnic group ☐
If Any Other Ethnic Group Specify	_____
Not stated	Not stated ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed:	**Print:**	**Date:**
Office use only, data entry completed by:		
Print Name:		**Date:**

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

APPENDIX 1 - GRADING CHART

All fields are mandatory

PVR Grade	Features
A	Vitreous haze; vitreous pigment clumps; pigment clusters on inferior retina
B	Wrinkling of inner retinal surface; retinal stiffness; vessel tortousity; rolled and irregular edge of retinal break; decreased mobility of vitreous
CA 1-12	Anterior to equator; focal, diffuse or circumferential full thickness folds; subretinal strands; anterior displacement; condensed viterous with strands
CP 1-12	Posterior to equator; focal, diffuse or circumferential full-thickness folds; subretinal strands; anterior displacement; condensed vitreous with strands

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Study No: ___ ___ ___

Appendix 2 - Visual Function Questionnaire – 25 (1/5)

Please answer all questions

Part 1 – General Health and Vision

1.	**In general**, would you say your overall **health** is*	*(Tick one)* Excellent ..... 1 ☐ Very Good ..... 2 ☐ Good ..... 3 ☐ Fair ..... 4 ☐ Poor ..... 5 ☐
2.	At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is excellent, good, **fair**, **poor**, or **very poor** or are you **completely blind**?	*(Tick one)* Excellent ..... 1 ☐ Good ..... 2 ☐ Fair ..... 3 ☐ Poor ..... 4 ☐ Very Poor ..... 5 ☐ Completely Blind ..... 6 ☐
3.	How much of the time do you **worry** about your eyesight?	*(Tick one)* None of the time ..... 1 ☐ A little of the time ..... 2 ☐ Some of the time ..... 3 ☐ Most of the time ..... 4 ☐ All of the time ..... 5 ☐
4.	How much **pain or discomfort** have you had **in and around your eyes** (for example, burning, itching, or aching)? Would you say it is:	*(Tick one)* None ..... 1 ☐ Mild ..... 2 ☐ Moderate ..... 3 ☐ Severe, or ..... 4 ☐ Very severe ..... 5 ☐

* Skip Question 1 when the VFQ-25 is administered at the same time as the SF-36 or RAND 36-Item Health Survey 1.0

The next questions are about how much difficulty, if any, you have doing certain activities wearing your glasses or contact lenses if you use them for that activity.

Part 2 – Difficulty with activities (Read categories as needed)

5.	How much difficulty do you have **reading ordinary print in newspapers**? Would you say you have:	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
6.	How much difficulty do you have doing work or hobbies that require you to **see well up close**, such as cooking, sewing, fixing things around the house, or using hand tools? Would you say:	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐

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Ozurdex in PVR Study

Study No: ___ ___ ___

Appendix 2 - Visual Function Questionnaire – 25 (2/5)

Please answer all questions

7.	Because of your eyesight, how much difficulty do you have finding **something on a crowded shelf**?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
8.	How much difficulty do you have **reading street signs or the names of stores**?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
9.	Because of your eyesight, how much difficulty do you have **going down steps, stairs, or curbs in dim light or at night**?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
10.	Because of your eyesight, how much difficulty do you have **noticing objects off to the side while you are walking along**?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
11.	Because of your eyesight, how much difficulty do you have **seeing how people react to things** you say?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
12.	Because of your eyesight, how much difficulty do you have **picking out and matching your own clothes**?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐

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Study No: ___ ___ ___

Appendix 2 - Visual Function Questionnaire – 25 (3/5)

Please answer all questions

13.	Because of your eyesight, how much difficulty do you have visiting **with people in their homes, at parties, or in restaurants**?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
14.	Because of your eyesight, how much difficulty do you have **going out to see movies, plays, or sports events**?	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
15.	Now, I'd like to ask about **driving a car**. Are you **currently driving**, at least once in a while?	*(Tick one)* Yes *(Skip to Q15c)* ..... 1 ☐ No ..... 2 ☐
15a.	IF NO, ASK: Have you **never** driven a car or have you **given up driving**?	*(Tick one)* Never drove *(Skip to Part 3, Q17)* ..... 1 ☐ No ..... 2 ☐
15b.	IF GAVE UP DRIVING: Was that **mainly because of your eyesight, mainly for some other reason**, or because of **both your eyesight and other reasons**?	*(Tick one)* Mainly eyesight *(Skip to Part 3, Q17)* ..... 1 ☐ Mainly other reason *(Skip to Part 3, Q17)* ..... 2 ☐ Both eyesight and other reason *(Skip to Part 3, Q17)* 3 ☐
15c.	IF CURRENTLY DRIVING: How much difficulty do you have **driving during the daytime in familiar places**? Would you say you have:	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐
16.	How much difficulty do you have **driving at night**? Would you say you have:	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐
16a.	How much difficulty do you have **driving in difficult conditions, such as in bad weather, during rush hour, on the freeway, or in city traffic**? Would you say you have:	*(Tick one)* No difficulty at all ..... 1 ☐ A little difficulty ..... 2 ☐ Moderate difficulty ..... 3 ☐ Extreme difficulty ..... 4 ☐ Stopped doing this because of your eyesight ..... 5 ☐ Stopped doing this for other reasons or not interested in doing this ..... 6 ☐

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Study No: ___ ___ ___

Appendix 2 - Visual Function Questionnaire – 25 (4/5)

Please answer all questions

The next questions are about how things you do may be affected by your vision. For each one, I'd like you to tell me if this is true for you all, most, some, a little, or none of the time.

Part 3 – Responses to Vision Problems

17.	**Do you accomplish less** than you would like because of your vision?	*(Tick one)* All of the time ..... 1 ☐ Most of the time ..... 2 ☐ Some of the time ..... 3 ☐ A little of the time ..... 4 ☐ None of the time ..... 5 ☐
18.	**Are you limited** in how long you can work or do other activities because of your vision?	*(Tick one)* All of the time ..... 1 ☐ Most of the time ..... 2 ☐ Some of the time ..... 3 ☐ A little of the time ..... 4 ☐ None of the time ..... 5 ☐
19.	How much does pain or discomfort **in or around your eyes**, for example, burning, itching, or aching, keep you from doing what you'd like to be doing? Would you say:	*(Tick one)* All of the time ..... 1 ☐ Most of the time ..... 2 ☐ Some of the time ..... 3 ☐ A little of the time ..... 4 ☐ None of the time ..... 5 ☐

For each of the following statements, please tell me if it is definitely true, mostly true, mostly false, or definitely false for you or you are not sure.

20.	**I stay home most of the time** because of my eyesight:	*(Tick one)* Definitely True ..... 1 ☐ Mostly True ..... 2 ☐ Not Sure ..... 3 ☐ Mostly False ..... 4 ☐ Definitely False ..... 5 ☐
21.	I feel **frustrated** a lot of the time because of my Eyesight:	*(Tick one)* Definitely True ..... 1 ☐ Mostly True ..... 2 ☐ Not Sure ..... 3 ☐ Mostly False ..... 4 ☐ Definitely False ..... 5 ☐
22.	I have **much less control** over what I do, because of my eyesight:	*(Tick one)* Definitely True ..... 1 ☐ Mostly True ..... 2 ☐ Not Sure ..... 3 ☐ Mostly False ..... 4 ☐ Definitely False ..... 5 ☐

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Study No: ___ ___ ___

Appendix 2 - Visual Function Questionnaire – 25 (5/5)

Please answer all questions

23.	Because of my eyesight, I have to **rely too much on what other people tell me**:	*(Tick one)* Definitely True ..... 1 ☐ Mostly True ..... 2 ☐ Not Sure ..... 3 ☐ Mostly False ..... 4 ☐ Definitely False ..... 5 ☐
24.	I **need a lot of help** from others because of my Eyesight:	*(Tick one)* Definitely True ..... 1 ☐ Mostly True ..... 2 ☐ Not Sure ..... 3 ☐ Mostly False ..... 4 ☐ Definitely False ..... 5 ☐
25.	I worry about **doing things that will embarrass myself or others, because of my eyesight**:	*(Tick one)* Definitely True ..... 1 ☐ Mostly True ..... 2 ☐ Not Sure ..... 3 ☐ Mostly False ..... 4 ☐ Definitely False ..... 5 ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed:	**Print:**	**Date:**
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Print Name:		**Date:**

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Ozurdex in PVR Study

Study No: ___ ___ ___

Appendix 2 - Health Survey – SF36 (1/3)

Please answer all questions

Health Survey

1.	**In general**, would you say your overall **health** is	*(Tick one)* Excellent ..... 1 ☐ Very Good ..... 2 ☐ Good ..... 3 ☐ Fair ..... 4 ☐ Poor ..... 5 ☐
2.	**Compared to ONE YEAR AGO**, how would you rate your health in general **NOW**?	*(Tick one)* MUCH BETTER than one year ago ..... 1 ☐ Somewhat BETTER now than one year ago ..... 2 ☐ About the SAME as one year ago ..... 3 ☐ Somewhat WORSE now than one year ago ..... 4 ☐ MUCH WORSE now than one year ago ..... 5 ☐

The following items are about activities you might do during a typical day. **Does your health now limit you** in these activities? If so, how much?

	Activities	Yes limited a lot	Yes, limited a little	No, not limited at all
3.	**Vigorous activities,** such as running, lifting heavy objects, participating in strenuous sports?	1 ☐	2 ☐	3 ☐
4.	**Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	1 ☐	2 ☐	3 ☐
5.	Lifting or carrying groceries?	1 ☐	2 ☐	3 ☐
6.	Climbing **several flights** of stairs?	1 ☐	2 ☐	3 ☐
7.	Climbing **one** flight of stairs?	1 ☐	2 ☐	3 ☐
8.	Bending, kneeing or stooping?	1 ☐	2 ☐	3 ☐
9.	Walking **more than a mile**?	1 ☐	2 ☐	3 ☐
10.	Walking **several** blocks?	1 ☐	2 ☐	3 ☐
11.	Walking **one** block?	1 ☐	2 ☐	3 ☐
12.	Bathing or dressing yourself?	1 ☐	2 ☐	3 ☐

During the **past 4 weeks**, have you had any of the following problems with your work or other regular activities *as a result of your physical health*?

		Yes	No
13.	Cut down on the **amount of time** you spent on work or other activities?	1 ☐	2 ☐
14.	**Accomplished less** than you would like?	1 ☐	2 ☐
15.	Were limited in the **kind** of work or other activities?	1 ☐	2 ☐
16.	Had **difficulty** performing the work or other activities (for example it took extra effort)?	1 ☐	2 ☐

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

		Yes	No
17.	Cut down on the **amount of time** you spent on work or other activities?	1 ☐	2 ☐
18.	**Accomplished less** than you would like?	1 ☐	2 ☐
19.	Didn't do work or other activities as **carefully** as usual?	1 ☐	2 ☐

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Study No: ___ ___ ___

Appendix 2 - Health Survey – SF36 (2/3)

Please answer all questions

20.	During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?	*(Tick one)* Not at all ..... 1 ☐ Slightly ..... 2 ☐ Moderately ..... 3 ☐ Quite a bit ..... 4 ☐ Extremely ..... 5 ☐
21.	How much **bodily pain** have you had during the **past 4 weeks**?	*(Tick one)* None ..... 1 ☐ Very Mild ..... 2 ☐ Mild ..... 3 ☐ Moderate ..... 4 ☐ Severe ..... 5 ☐ Very Severe .....
22.	During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?	*(Tick one)* Not at all ..... 1 ☐ Slightly ..... 2 ☐ Moderately ..... 3 ☐ Quite a bit ..... 4 ☐ Extremely ..... 5 ☐

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the **past 4 week …**

		All of the time	**Most of the time**	**A good bit of the time**	**Some of the time**	**A little of the time**	**None of the time**
23.	Did you feel full of pep?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
24.	Have you been a very nervous person?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
25.	Have you felt so down in the dumps that nothing could cheer you up?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
26.	Have you felt calm and peaceful?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
27.	Did you have a lot of energy?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
28.	Have you felt downhearted and blue?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
29.	Do you feel worn out?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
30.	Have you been a happy person?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐
31.	Did you feel tired?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐	6 ☐

32.	During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?	*(Tick one)* All of the time ..... 1 ☐ Most of the time ..... 2 ☐ Some of the time ..... 3 ☐ A little of the time ..... 4 ☐ None of the time ..... 5 ☐

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Appendix 2 - Health Survey – SF36 (3/3)

Please answer all questions

How TRUE or FALSE is **each** of the following statements for you?					
	Definitely True	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
33. I seem to get sick a little easier than other people?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
34. I am as healthy as anybody I know?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
35. I expect my health to get worse?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
36. My health is excellent?	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

I can confirm that I have completed this form in full and take full responsibility for any missing data.

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Study No: ___ ___ ___

Appendix 2 - Depression Screener (1/1)

Please answer all questions

Almost everyone has experienced times of feeling sad or depressed, like when suffering from a severe illness, when a person close to you has died, or if there are problems at work or in the family. The following questions are about such times.

Patient Questionnaire		
1.	Have you ever had **2 years or more** in your life when you felt depressed or sad most days, even if you felt OK sometimes?	*(Tick one)* Yes ..... 1 ☐ No (Skip to Question 2) ..... 2 ☐
a.	Did any period like that ever last 2 years without an interruption of 2 full months when you felt OK?	*(Tick one)* Yes ..... 1 ☐ No (Skip to Question 2) ..... 2 ☐
b.	Did any of those long periods of feeling sad or depressed continue into the last 12 months?	*(Tick one)* Yes ..... 1 ☐ No ..... 2 ☐
2.	In the last **12 months**, have you had **2 weeks or longer** when ...	
a.	nearly every day you felt sad, empty or depressed for most of the day?	*(Tick one)* Yes ..... 1 ☐ No ..... 2 ☐
b.	you lost interest in most things like work, hobbies, and other things you usually enjoyed?	*(Tick one)* Yes ..... 1 ☐ No ..... 2 ☐
3.	In the **last month** did you have a period of **1 week or more** when ...	
a.	nearly every day you felt sad, empty or depressed for most of the day?	*(Tick one)* Yes ..... 1 ☐ No ..... 2 ☐
b.	you lost interest in most things like work, hobbies, and other things you usually enjoyed?	*(Tick one)* Yes ..... 1 ☐ No ..... 2 ☐

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Study No: ___ ___ ___

APPENDIX 3 - UNSCHEDULED VISIT ASSESSMENT FORM

Study Eye Data Only, all fields are mandatory

Examination Details

Missed Visit? ☐

Date of Visit *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
ETDRS VA *(Enter no. of letters, range 0-100)* If score '0' please specify	__ __ __ CF ☐ HM ☐ PL ☐ NPL ☐
IOP mmHG *(Range – 0-80)*	__ __

		Yes	No	NP
AC *(Tick yes or no)*	Uveiitis	☐	☐	☐
	Fibrin	☐	☐	☐
	Hyphaema	☐	☐	☐
	Oil (patent PI)	☐	☐	☐
	Oil (blocked PI)	☐	☐	☐
	Rubeosis	☐	☐	☐
	Corneal Oedema	☐	☐	☐
Lens *(Tick yes or no)*	Clear	☐	☐	☐
	AC IOL	☐	☐	☐
	PC IOL	☐	☐	☐
	Aphakic	☐	☐	☐
	Cataract	☐	☐	☐
State of Macula *(Tick yes or no)*	Attached	☐	☐	☐
	Detached	☐	☐	☐
	Oedema	☐	☐	☐
	Pucker	☐	☐	☐
	FTMH	☐	☐	☐
State of Retina *(Tick yes or no)*	Fully Attached	☐	☐	☐
	Traction RD	☐	☐	☐
	RRD	☐	☐	☐
	Open Previous Break	☐	☐	☐
	Entry Site Break	☐	☐	☐
	Other New Breaks	☐	☐	☐
	Visible Flat ERM	☐	☐	☐

If Cataract, state LOCS II	NS	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐
	CLO	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐	5+ ☐
	PSC	0 ☐	1+ ☐	2+ ☐	3+ ☐	4+ ☐

Other Procedures Since Last Visit

	Yes	No	*(dd mm yyyy)*		Yes	No	*(dd mm yyyy)*
Oil Out	☐	☐	__ __ / __ __ / __ __ __ __	Buckle	☐	☐	__ __ / __ __ / __ __ __ __
ICCE	☐	☐	__ __ / __ __ / __ __ __ __	Peel	☐	☐	__ __ / __ __ / __ __ __ __
E/C + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Retinectomy	☐	☐	__ __ / __ __ / __ __ __ __
Phako + IOL	☐	☐	__ __ / __ __ / __ __ __ __	Laser	☐	☐	__ __ / __ __ / __ __ __ __
YAG PI	☐	☐	__ __ / __ __ / __ __ __ __	Surgical PI	☐	☐	__ __ / __ __ / __ __ __ __
Other	☐	☐	__ __ / __ __ / __ __ __ __	*If Other Specify*			_____

OCT	Yes ☐	No ☐	NP ☐
If Yes Specify	Macular SRF	Yes ☐	No ☐
	Foveal Thickness *(Range 0-999)*	__ __ __ µm	NP ☐
	Macular Volume *(Range 0-100)*	__ __ . __ __ mm^3	NP ☐
PVR Present	Yes ☐		No ☐
If Yes Specify Grade (range 1-12)	CA ☐ __ __		CP ☐ __ __
Hypotensive Agents	Yes ☐		No ☐
If Yes Specify	Prostaglandin ☐	Topical CAI ☐	Combigan ☐
	B Blocker ☐	Systemic CAI ☐	Combined B Blocker + Prostaglandin ☐
	Alpha Agonist ☐	Cosopt ☐	

Retina Reattached without further VR surgical intervention	Yes ☐	No ☐
Silicone Oil In situ	Yes ☐	No ☐
Number of additional VR procedures since first vitrectomy apart from ROSO		__ __

I can confirm that I have completed this form in full and take full responsibility for any missing data.

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Ozurdex in PVR Study

Study No: ___ ___ ___

APPENDIX 4 - PROTOCOL DEVIATIONS FORM

All fields are mandatory

Deviation Details	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	
Deviation Date *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Type of Deviation	
Any Outcomes or Actions	

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Study No: ___ ___ ___

APPENDIX 5 - CONCOMITANT MEDICATION FORM PRIOR TO TRIAL

All fields are mandatory

Name of Medication	**Total Dose** per day	Approximate **Start Date** of medication	**Stop Date** *(leave blank if continuing on the medication)*

Comments

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Study No: ___ ___ ___

APPENDIX 6 - CONCOMITANT MEDICATION FORM DURING THE TRIAL

All fields are mandatory

Name of Medication	**Total Dose** per day	Approximate **Start Date** of medication	**Stop Date** *(leave blank if continuing on the medication)*

Comments

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APPENDIX 7 – EARLY STUDY WITHDRAWAL FORM

All fields are mandatory

Withdrawal Details	
Did the patient discontinue the trial prematurely?	Yes ☐ No ☐
Date of premature Study Discontinuation *(dd mm yyyy)*	__ __ / __ __ / __ __ __ __
Primary reason for discontinuation *(tick one box only)*	Patient withdraws consent ☐ *If known, state reason:* _____ Patient is participating in another trial ☐ Patient is non-compliant ☐ Patient is pregnant ☐ Patient is lost to follow up ☐ Investigator feels that it is in the patients best interest due to adverse event ☐ *Related AE No:* _____ Other reason for discontinuation ☐ *If Other specify:* _____
Does the patient still agree to have their data collected and analysed as part of an intent to treat analysis?	Yes ☐ No ☐

Comments

I can confirm that I have completed this form in full and take full responsibility for any missing data.

Signed:	**Print:**	**Date:**
Office use only, data entry completed by:		
Print Name:		**Date:**

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APPENDIX 8 - ADVERSE EVENTS FORM

Study No: ___ ___ ___

All fields are mandatory

Please enter details of adverse events below and use relevant codes where necessary.

Expected / Unexpected *(enter 'E' or 'U')*	Code	Start Date	Stop Date *(enter 'C' if continued)*	Severity	Study Drug Relationship	Action Taken Regarding Study Drug	Outcome of Adverse Event	Was this an SAE?	Initials	Date Recorded

ADVERSE EVENT CODES

Expected:
1 = Cataract
2 = Raised Intraocular Pressure
3 = Hypotony
4 = Sterile Hypopyon
5 = Retinal Detachment
6 = Uveitis
7 = Further Surgery
8 = Glaucoma
9 = Headache
10 = Migraine
11 = Vitreous Opacities
12 = Tractional Maculopathy

Unexpected:
13 = Endophthalmitis
14 = Systemic Illness
15 = Ocular Vascular Occlusion
16 = Other

Severity:
1 = Not Severe
2 = Moderate
3 = Severe

Study Drug Relationship:
1 = Not Related
2 = Possibly/Probably Related

Action Taken Regarding Study Drug:
1 = None
2 = Discontinued Permanent
3 = Discontinued Temporary
4 = Reduced Dose
5 = Increased Dose

Was this an SAE?
1 = Not Severe
2 = SAE
3 = SUSAR

Outcome of Adverse Event:
1 = Resolved without effects
2 = Resolved with effects
3 = On-going
4 = Death
5 = Unknown

Moorfields Eye Hospital NHS
NHS Foundation Trust

Ozurdex in PVR Study

Pharmacovigilance Adverse Event Log

Study No: ___ ___ ___

Visit Number	Visit Date	Any Adverse Events since their last study visit
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐
	__ __/__ __/__ __ __ __	*(If yes complete separate adverse event form)* Yes ☐ No ☐

Moorfields Eye Hospital NHS
NHS Foundation Trust

Date__ __ / __ __ / __ __ __ __ **Study No:** ___ ___ ___ ___

Ozurdex in PVR Study

PHARMACOVIGILANCE ADVERSE EVENT FORM

Site number:	Sponsor ID:	Eudract Number:	Study Drug:	Patient Initials:	Visit Number:
MEH/001	CHAD1030	2011-004498-96	Ozurdex		

Adverse Event Ocular/Non-ocular	Severity	Study Drug Relationship	Action Taken Regarding Study Treatment	Outcome of AE	Expected	Serious
L=event in left eye R=event in right eye B=event in both eyes N=Other non-ocular event (code each prefix/ non event)	1=Mild 2=Moderate 3=Severe	1=Definitely 2=Probably 3=Possibly 4=Unlikely 5=Not Related 6=Not Assessable	1=None 2=Discontinued permanently 3=Discontinued temporarily 4=Reduced dose 5=Increased dose 6=Delayed dose	1=Resolved, No Sequel 2=AE still present- no treatment 3=AE still present- being treated 4=Side effects present- not treated 5=Side effects present- treated 6=Death 7=Unknown	1=Yes 2=No	1=Yes 2=No ***(If yes complete SAE form)***

Adverse Event	Start Date	Stop Date	Severity	Relationship to Study Treatment	Action Taken with Study Treatment	Outcome of AE	Expected	Serious *(If yes complete SAE form)*	Initials
1.									
2.									
3.									

8.5 Appendix 5 Ozurdex in PVR Participant Information Leaflet

PARTICIPANT INFORMATION SHEET

(Version 3.2 – March 11th 2013)

EXPLANATION TO PATIENT

Title of Project : Ozurdex in proliferative vitreoretinopathy: a randomised controlled trial

Researcher: Mr. D.G. Charteris.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of this study?

The purpose of this study is to investigate the potential benefit of an additional anti-inflammatory treatment to improve the outcome of surgery in eyes that have developed scar tissue on the surface of the retina (proliferative vitreoretinopathy, PVR). The retina is a thin layer which lines the inside of the eye. It is sensitive to light (like the film in a camera) and is necessary for vision. If a hole or holes develop in a retina it can become detached. Your retina has detached and has developed scar tissue (PVR) which increases your risk of it detaching again after surgery. This is because the scar tissue pulls on the retina preventing the holes from being repaired by the standard technique used in other retinal detachment patients.

If the retina remains detached you will lose vision.

The study is designed to investigate whether this anti-inflammatory medication is a feasible treatment to prevent this scarring response from recurring.

The only treatment available is an operation which attempts to remove the scar tissue and close the holes, allowing the retina to reattach. The success rate is limited because the scar tissue often returns, leading to re-detachment.

Inflammation is the medical term used to describe the way in which the body reacts to injury. There is laboratory evidence that the formation of scar tissue might be prevented by using anti-inflammatory medications around the time of surgery. This anti-inflammatory medication is currently used routinely on patients in other clinical settings however, we do not yet know whether this additional treatment would be helpful in your situation. If you chose to enter the study we will not ask you to take any other additional medications as the treatment is given at the time of surgery.

Why have I been chosen?

You have been chosen because the retina in your eye has detached and has begun to develop scar tissue (PVR), and we are planning to carry out an operation to the back of your eye (vitreous gel and retina) to repair this. Initially we hope to study 140 patients with this condition.

What will happen to me if I take part?

The treatments are either the best available current therapy (standard treatment) or the study treatment. You will be randomly allocated to one of these treatments. Therefore you have a 50% chance of receiving the study treatment and 50% chance of receiving the standard treatment.

Study Treatment:

The operation will be the standard one for your particular type of retinal detachment. At the time of surgery we will peel away areas of scar tissue and treat any retinal holes by 'spot welding' them back into place. In addition to the standard operation we plan to treat the eye at the time of surgery with an anti-inflammatory drug called **OZURDEX**. This drug will be administered as a local injection into the eyeball. This will be done whilst you are still under anaesthetic so you will not be aware of the additional treatment.

Following the surgery you will be given the standard steroid, antibiotic and pupil dilating eye drops that are usually prescribed following this type of surgery.

As is routine for patients undergoing surgery for retinal detachments with scar tissue, silicone oil is put into the eye at the time of the operation to help the retina stay in place. This is normally removed approximately 3-6 months later. We plan to treat the eye with another injection of **Ozurdex** at the time of this second procedure.

Standard Treatment:

The operation will be the standard one for your particular type of retinal detachment. At the time of surgery we will peel away areas of scar tissue and treat any retinal holes by 'spot welding' them back into place. Following the surgery you will be given the standard steroid, antibiotic and pupil dilating eye drops that are usually prescribed following this type of surgery. As is routine for patients undergoing surgery for retinal detachments with scar tissue, silicone oil is put into the eye at the time of the operation to help the retina stay in place. This is normally removed approximately 3-6 months later.

What will happen to me at each clinic visit?

By participating in this study, you will be asked to visit the clinic for 1 additional visit lasting approximately 10-15 minutes in the first 6 months and we will continue to see you for 2 further appointments at 9 months and 12 months after the initial surgery.

At each visit, your vision will be checked and the doctor will examine your eye as usual with a microscope. You will also have a routine scan (OCT scan) of the back of your eye. This is completely painless and harmless and does not involve radiation.

You will be asked to complete a short questionnaire at the beginning, middle and end of the trial. This will ask questions about how your eyesight affects your life and your mood.

What are the drugs that are being tested?

OZURDEX is a type of steroid that is commonly used in the treatment of inflammation in a number of eye conditions and in eyes that have suffered blood vessel blockages. It has been shown to be a safe and effective drug when administered into the eye. In this study the OZURDEX is being used outside the terms of its license.

What are the side effects of taking part?

The medicine that will be used is not new, and has been extensively used for other medical conditions. Therefore we do not expect to discover unknown side effects.

OZURDEX may cause the pressure in your eye to increase, however we can treat this with pressure lowering drops. The pressure usually returns to normal once the drug's effect has stopped. If the pressure in your eye remains increased above a certain level, your doctor will not give you the second injection of OZURDEX.

If during the study there is strong evidence from other studies that the use of OZURDEX results in patients having a poorer outcome than conventional treatment we will stop the study.

What are the possible benefits of taking part?

We hope that the treatment will help you. However this cannot be guaranteed. The information we get from this study may help us to treat future patients with retinal detachment by improving the success rate of the surgery.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive. If you do not wish to take part in this study you will not be at a disadvantage and will continue to receive normal clinical management.

What if I become pregnant whilst taking part in the study?

We shall not ask you to take part in the study if you are pregnant or breast-feeding, and if you are a female of child-bearing age, we shall ask you to take a pregnancy test to confirm that you are not pregnant. We shall also ask you to agree to adequate contraception throughout the duration of the trial (12 months).

If you do become pregnant during the trial, we shall refer you back to your GP to monitor your pregnancy routinely, as there is no evidence to suggest that the trial medication is harmful to you or your unborn baby. We will ask you to continue to be reviewed as part of the trial if you agree.

What happens when the research study finishes?

If you like, we can tell you which study group you were in. If you still require follow-up for your eye condition, this will be continued routinely in the out-patient department, and not as part of the study in the clinical trials unit.

What will happen to the results of the study?

We plan to publish the results of this study in a medical journal, and if you like, we can give you information about how to access this material. Please remember that as there are 140 participants in the study, the process may take many years before this information is available.

What if something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms may be available to you.

Who is organising and funding the research?

This study is a **commercially funded** but **investigator led** trial. This means that the funding for the trial is being provided by Allergan Ltd (the pharmaceutical company which makes OZURDEX) but the organisation and clinical running of the trial is the responsibility of the investigating research team at Moorfields Eye Hospital.

Will my taking part in this study be kept confidential?

Information derived from the study will be treated as completely confidential. Information will be stored in electronic and paper form and kept in a secure location. All electronic data storage

will comply with the requirements of the data protection act. Your GP will be informed of your participation in this study.

Who has reviewed the study?

A full scientific protocol for this research has been approved by the National Research Ethics Committee London – Central. This study complies and at all times will comply with the Declaration of Helsinki[1] as adopted at the 52nd WMA General Assembly, Edinburgh, October 2000 and with the Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research, (Strasbourg 25.1.2005). Ask the Project Manager if you would like further details of the approval or to see a copy of the full protocol.

Contact Numbers:

Emergency Contact:

You will be given a copy of this information sheet and the consent form, which you have signed, to keep. The original will be retained in your clinical notes.

[1] World Medical Association (2000) Declaration of Helsinki. Ethical principles for medical research involving human subjects. 52nd World Medical Association General Assembly, Edinburgh, Scotland October 2000.

8.6 Appendix 6 Ozurdex in PVR Study GP Letter

Dear Dr

This is to inform you that your patient:

has been recruited into a new study at Moorfields Eye Hospital entitled:

Ozurdex in proliferative vitreoretinopathy: a randomised control trial

Please find enclosed a copy of the Participant Information Sheet.

Your patient has given written consent to take part in this study. They are of course free to withdraw at any time, without needing to give a reason. The study has been subject to a review by The Research Ethics Committee London Central, who have given approval for the study to take place.

If you require any further information or you feel that your patient is unsuitable to take part in this trial, please do not hesitate to contact us.

Yours sincerely

8.7 Appendix 7 Ozurdex in PVR Consent Form

CONSENT FORM

Study Title: Ozurdex in proliferative vitreoretinopathy: a randomised control trial

Researcher: Mr D. G. Charteris

Please initial box

I confirm that I have read and understand the information sheet dated March 11th 2013 version number 3.2 for the above study and have had the opportunity to ask questions ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

I understand that sections of my medical notes may be looked at by responsible individuals from Moorfields Eye Hospital or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records ☐

I understand that my GP will be informed of my participation in this research project and of any findings significant to my general health ☐

I agree to take part in the above study ☐

_____	_____	_____
Name of patient	**Date**	**Signature**
_____	_____	_____
Name of person taking consent (if different from researcher)	**Date**	**Signature**
_____	_____	_____
Researcher	**Date**	**Signature**

8.8 Appendix 8 Stem cell feasibility study - Participant Information Leaflet

Investigation into the feasibility of harvesting Stem Cells from Adult Human Retina Tissue; The StRetTis Project

Principal Investigator: Mr Philip Banerjee

Co-Investigators: Mr Hari Jayaram, Dr Astrid Limb, Mr David Charteris

We would like to invite you to take part in a research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have.

Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear.

You are being invited to take part in this research study because you are undergoing retinal surgery under the vitreoretinal service at Moorfields Eye Hospital. Before you decide to participate it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully. Talk to others about the study if you wish.

Part 1

What is the purpose of the study?

Stem cells (cells capable of dividing to produce numerous other cell types) are known to exist within the adult human retina. In the future, cells such as these may potentially be used to develop new treatments for individuals who have lost vision from retinal disease. We know that it is possible to readily obtain such cells from large samples of retina and the aim of this study is to see if it is possible to obtain cells from very small samples of retina.

Why have I been invited?

You have been invited as you are due to undergo retinal surgery at Moorfields Eye Hospital. Your surgical procedure will involve the removal of very small amount of tissue from the outer retina which is usually discarded at the end of the procedure.

Do I have to take part?

It is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What will happen to me if I take part?

We would ask you to consider donating the very small fragments of retinal tissue for use in the laboratory at the UCL Institute of Ophthalmology, which would be normally removed and discarded as part of the surgical treatment. Other than this, there is no further involvement required on your part.

Expenses and payments

There will no expenses provided for this study, as no additional visits to the hospital are necessary. If you give your consent to donate a sample of retinal tissue, it will be treated as a gift, and as such you will be giving up all legal rights to the sample and will not be eligible for payment should the laboratory experiments lead to the development of new treatments in years to come.

What will I have to do?

One of our team will go through the patient information sheet with you and answer any further questions you may have and obtain your consent. Other than this your operation will take place as normal, except that we shall collect the small samples of retina during the procedure that would normally be discarded.

What are the possible disadvantages and risks of taking part?

There are no specific risks to taking part in this study as we shall be collecting tissue that would normally be discarded. Your clinical team will have discussed the risks associated with the surgical procedure with you on a separate occasion before your operation.

What are the possible benefits of taking part?

We cannot promise the study will help you directly but the information we get from this study will help medical scientists to try to develop new treatments for retinal disease in the future.

What happens when the research study stops?

Your participation in this study will not affect your clinical care during the study or after its completion. The findings of the study will have an influence upon the development of future work towards new treatments for retinal disease.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes we get new information about the subject being studied. If this happens, your research doctor will tell you and discuss whether your participation is required in the study. However we would expect this to be very unlikely

What will happen if I don't want to carry on with the study?

You can withdraw from the study at anytime without your clinical care being affected.

What if there is a problem?

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Moorfields Eye Hospital NHS Foundation Trust, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you

Will my taking part in this study be kept confidential?

Your samples will be fully anonymised using a unique identification number and researchers will not be able to identify the donor. No identifiable data will be used or stored for the purpose of this study.

What will happen to any samples I give?

Your tissue sample will be immediately sent to the laboratory in the UCL Institute of Ophthalmology, where we will attempt to isolate stem cells. The process of cell isolation involves the use of enzymes to break down the tissue and therefore there will be no tissue remaining after processing. Any cells that are grown in culture will be studied in the laboratory, and will not be used for any experiments involving animals. Any cells that remain at the end of the study period will be destroyed.

What will happen to the results of the research study?

The results of the study will be published in a scientific journal or be presented at a conference. All data will be anonymous and none of the participants involved the study can be identified from any reports published. Should you wish to see the results of the study in the future, please contact the study doctor.

Who is organising and funding the research?

This project is being sponsored by Moorfields Eye Hospital NHS Foundation Trust with support from the NIHR Biomedical Centre for Ophthalmology at Moorfields Eye Hospital and the UCL Institute of Ophthalmology.

Who has reviewed the study?

This study has been reviewed and given a favourable opinion by the National Research Ethics Service (NRES) committee, East Midlands.

Further information and contact details

For any further information, please contact the principal investigator, Mr Philip Banerjee, Vitreoretinal Research Fellow on 020 75662283 or Philip.Banerjee@moorfields.nhs.uk . If you have any concerns regarding the conduct of the study, please contact the Patient Advice and Liaison Service (PALS) on 0207 253 3411 extension 2325

8.9 Appendix 9 Stem cell feasibility study – Consent Form

CONSENT FORM

Study Title: Investigation into the feasibility of harvesting Stem Cells from Human Retinal Tissue; The StRetTis Project

Researcher: Mr P J Banerjee **Please initial box**

1. I confirm that I have read and understand the information sheet dated 17th February 2012 version number 1.2 for the above study and have had the opportunity to ask questions ☐
2. I agree to donate a sample of retinal tissue (that would otherwise be discarded) as a gift for research in the above project. I understand how the sample will be collected and that giving a sample is voluntary. ☐
3. In giving this sample as a gift, I understand that I am giving up all legal rights with regards to its use, and will not be eligible for payment should the experiments lead to any new intellectual property. ☐
4. I understand that the sample I have given will be sent to a laboratory in the UCL Institute of Ophthalmology, as described in the patient information sheet ☐
5. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor of the study (Moorfields Eye Hospital NHS Foundation Trust) and the regulatory agencies, where it is relevant to my participation in this study. I give permission for these individuals to have access to my records. ☐

_____	_____	_____
Name of patient	**Date**	**Signature**
_____	_____	_____
Name of person taking consent (if different from researcher)	**Date**	**Signature**
_____	_____	_____
Researcher	**Date**	**Signature**